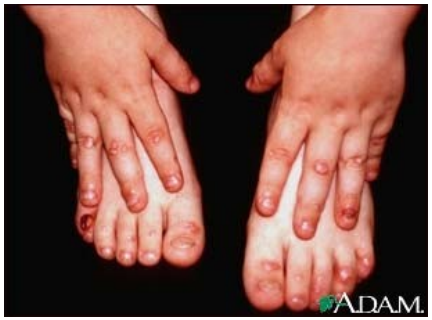
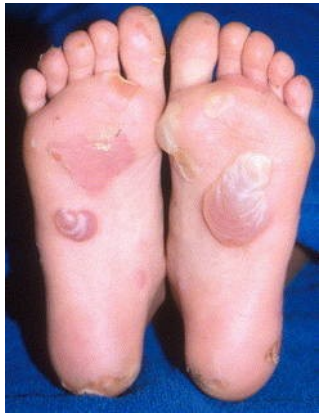
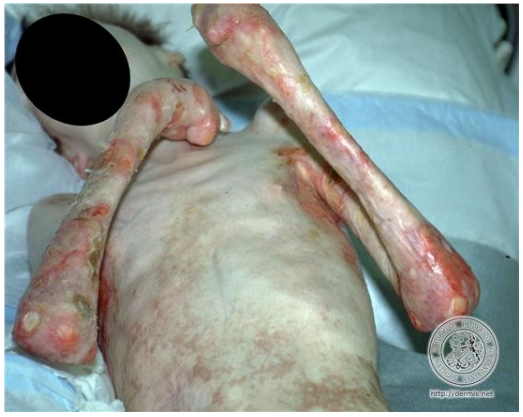
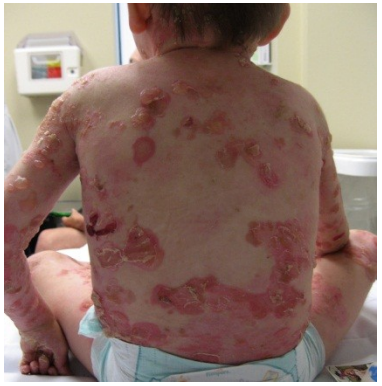
- Epidermolysis bullosa
- Inherited ichthyoses

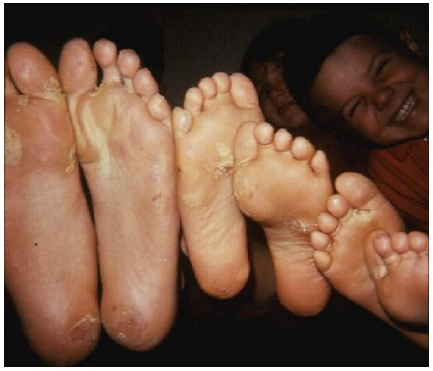
- Group of diseases sharing two characteristic features - **fragility of the skin** and **blistering.**
- **The clinical features have a broad range of severity** - from isolated nail dystrophy through relatively mild, localised blistering of extremities to generalized blistering and mutilation; adding to cutaneous complexity is finding of extracutaneous manifestations.

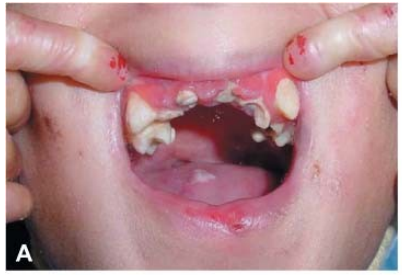


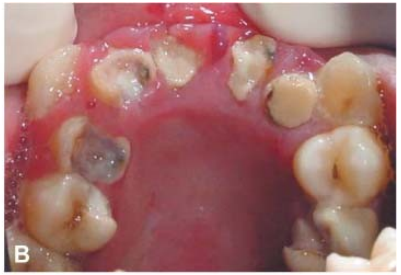












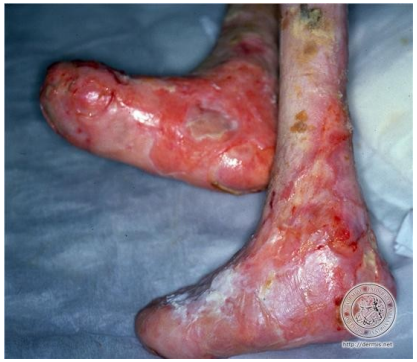
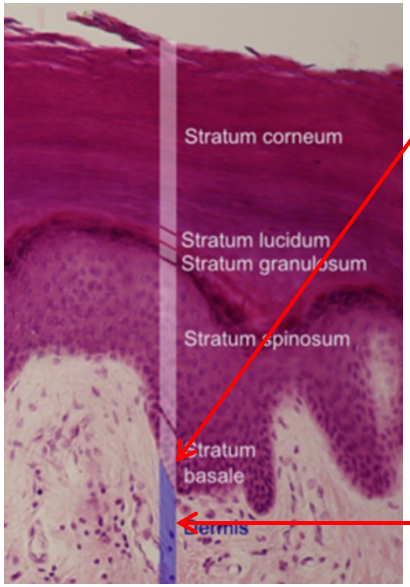


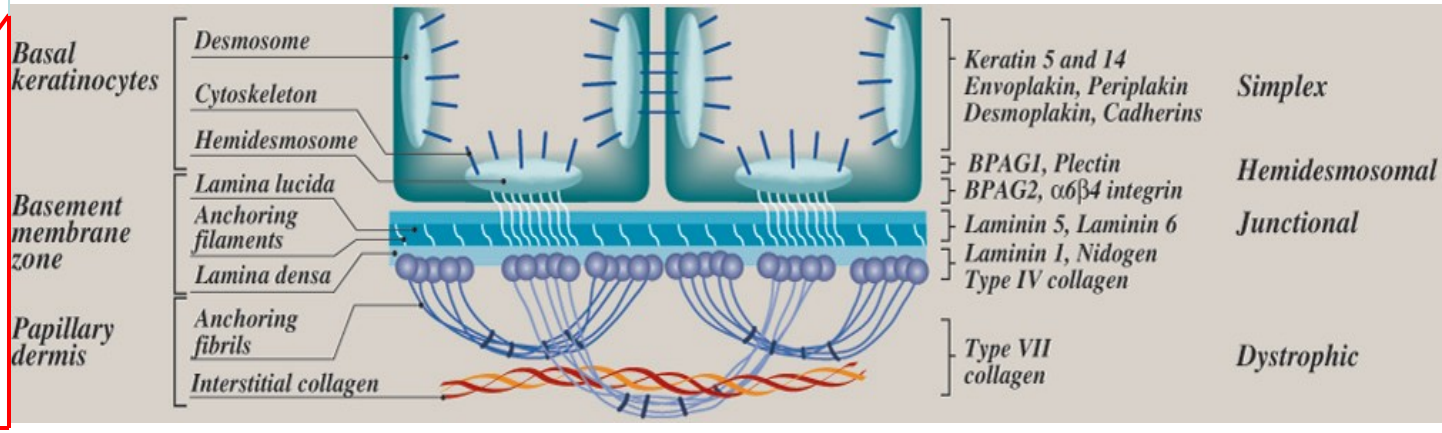
FIGURE 1- A= Frontal view of open mouth with patient's help. The limitation of mouth opening was a consequence of microstomia. B = Maximal upper occlusal view

- **EB simplex** – vznik puchýřů na úrovni bazálních keratinocytů – ***KRT5* (keratin 5), *KRT14* (keratin 14)**, *DSP* (desmoplakin), *DST* (dystonin), *JUP* (junction plakoglobin), *PKP1* (plakophilin), *EXPH5* (exophilin 5), *PLEC* (plectin), ***TGM5* (transglutaminase 5)**

- **EB junkční** – vznik puchýřů na úrovni bazální membrány – *COL17A1* (collagen, type XVII, alpha 1), *LAMA3* (laminin, alpha 3), *LAMB3* (laminin, beta 3), *LAMC2* (laminin, gamma 2), *ITGA6* (integrin, alpha 6), *ITGB4* (integrin, beta 4), *ITGA3* (integrin, alpha 3)

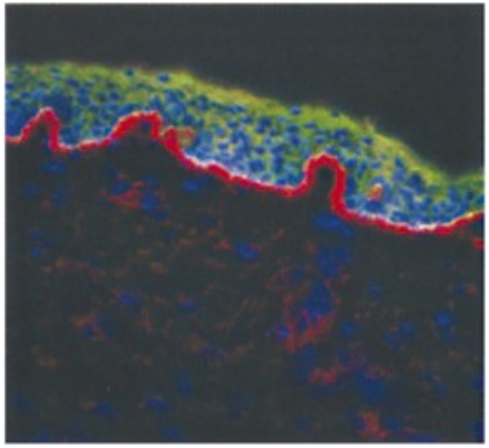
- **EB dystrofická** – vznik puchýřů pod bazální membránou, na úrovni kotvících fibril – ***COL7A1* (collagen, type VII, alpha 1)**



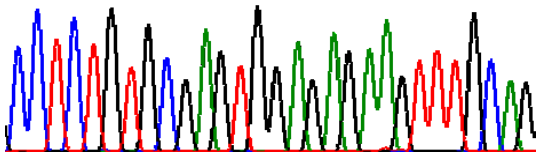


- In 1960s, **transmission electron microscopy** was first applied to subdivide EB into three main categories.

- In 1980s, the discovery and development of **antibodies** to different skin proteins permitted further insight into the pathophysiology of EB by **immunofluorescence mapping of protein**.

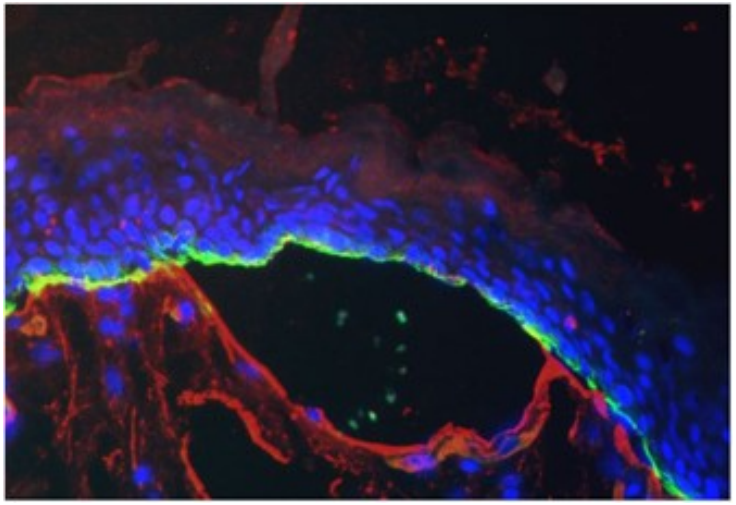


- In 1990s, **molecular genetic analysis** of genes associated with particular types of EB was introduced.

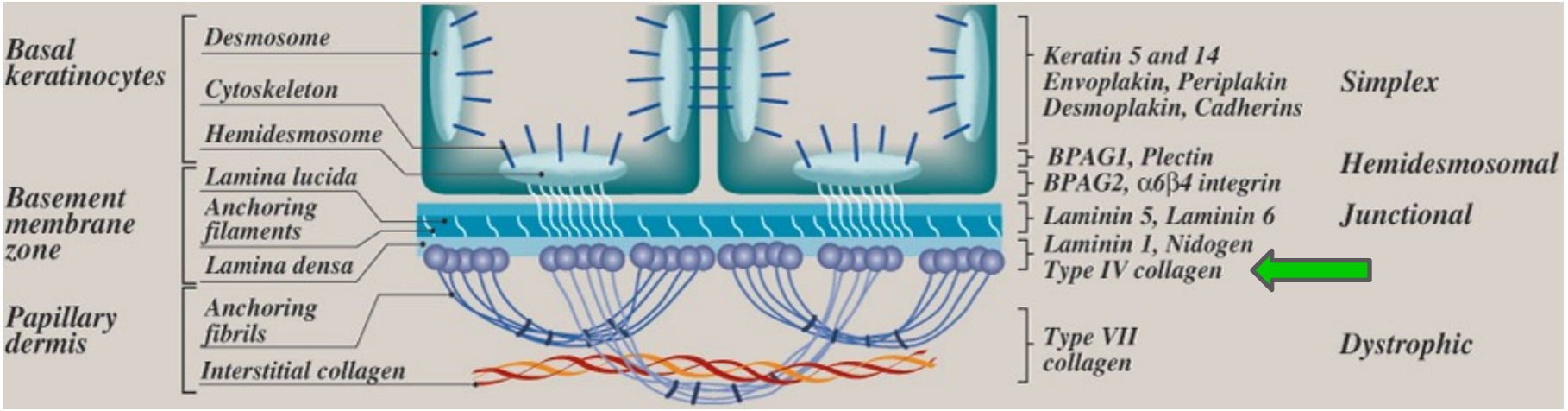


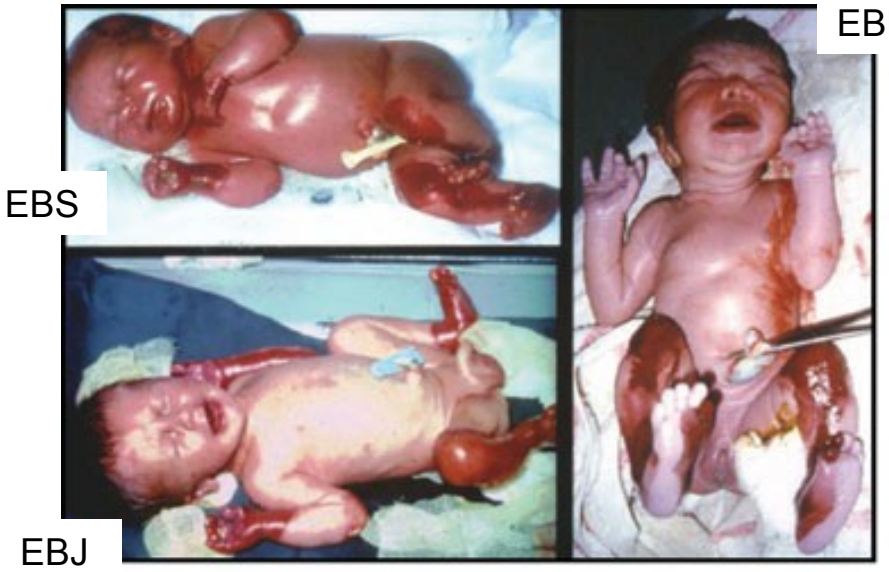
- **Mapping of type IV collagen** (the lamina densa) **to the roof of a blister** in an EB patient's skin, provided a rapid diagnosis of **dystrophic EB**.

- **Mapping of type IV collagen to the floor of blister** in an EB patient s skin, provided diagnosis of **EB simplex or junctional EB**.

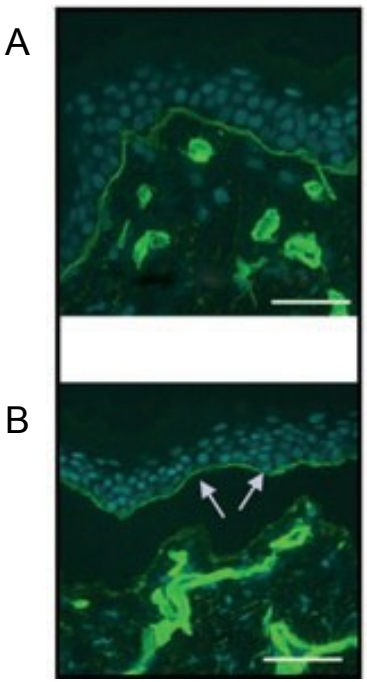


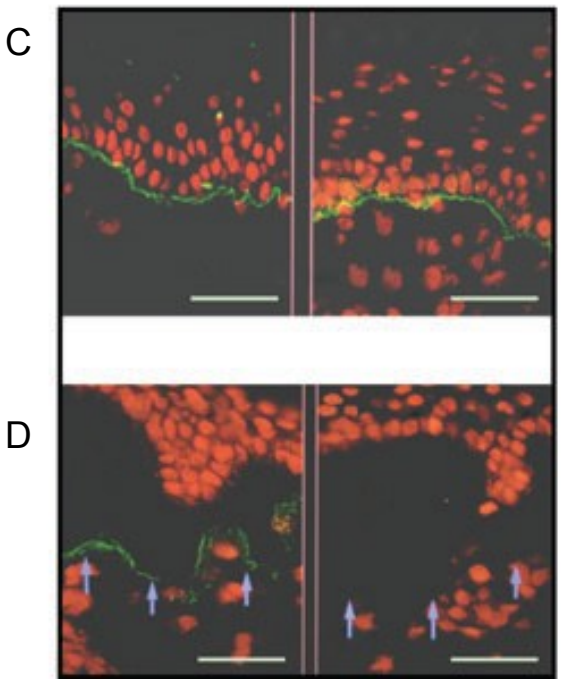
Focal separation within the epidermal basement membrane delineated by type IV collagen (stained red) and bullous pemphigoid antigen 2 (stained green). Nuclei (counterstained blue) outline the overlying epidermis as well as selected cells in the dermis.

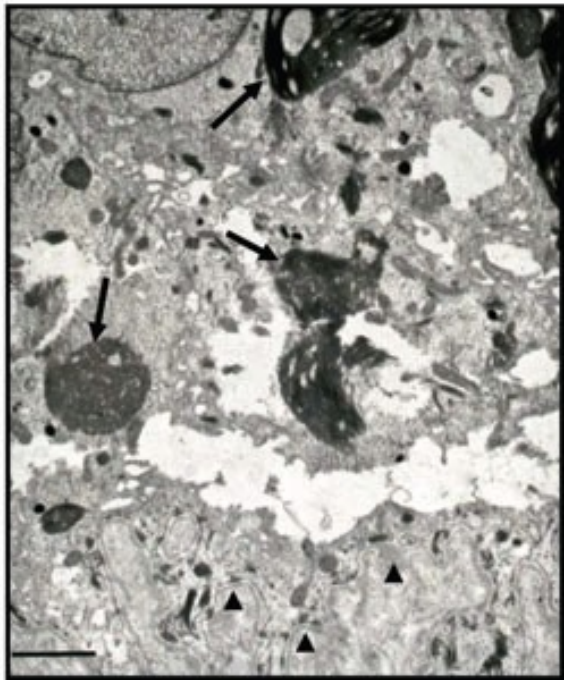




A) normal skin, antibody to the type IV collagen, staining at the dermal–epidermal junction. B) patient, naturally occurring blister and the dermal–epidermal junction, labelling maps to the roof of the split, indicating sublamina densa plane of cleavage → EBD. C) normal skin, antibodies to the laminin, linear immunoreactivity at the dermal–epidermal junction. D) patient, the laminin labelling is present but maps to the base of the split → EBJ. G) Diagnosis of Dowling–Meara EB simplex by transmission electron microscopy.



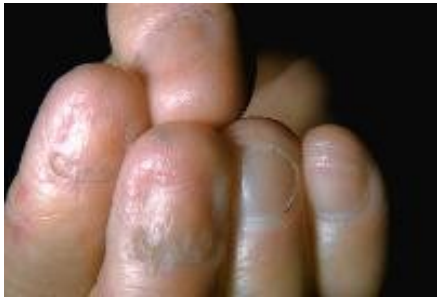


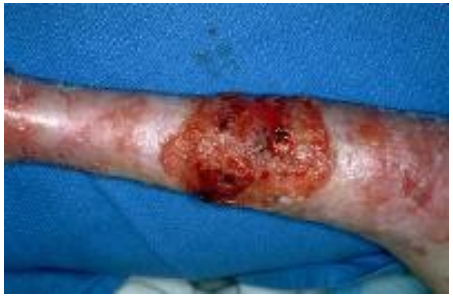


Histopathology 2010, 56.

1. Klinické příznaky

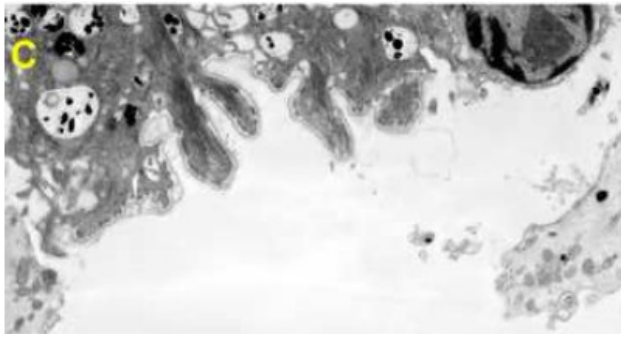
(*FNB, Pediatrická klinika*)

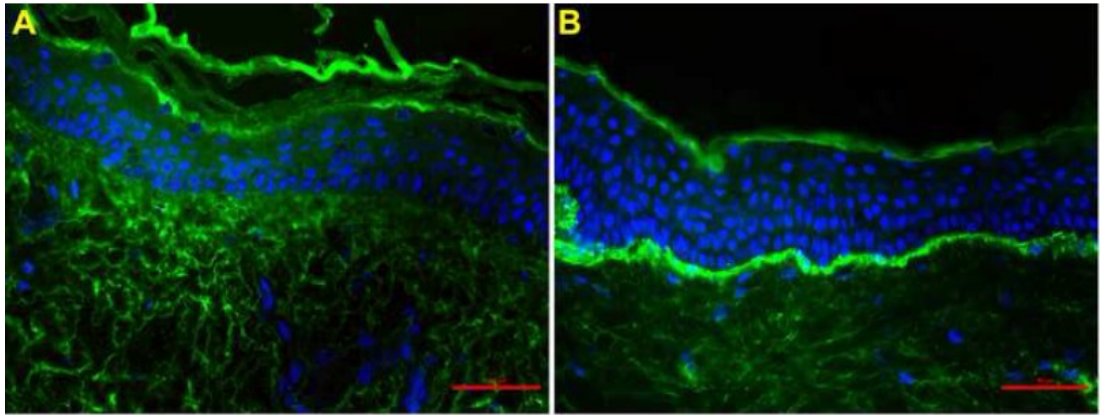




2. Transmisní elektronová mikroskopie

(*FNuSA, PAU*)



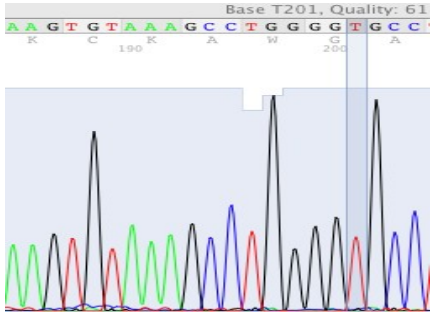


3. Imunofluorescenční antigenní mapování

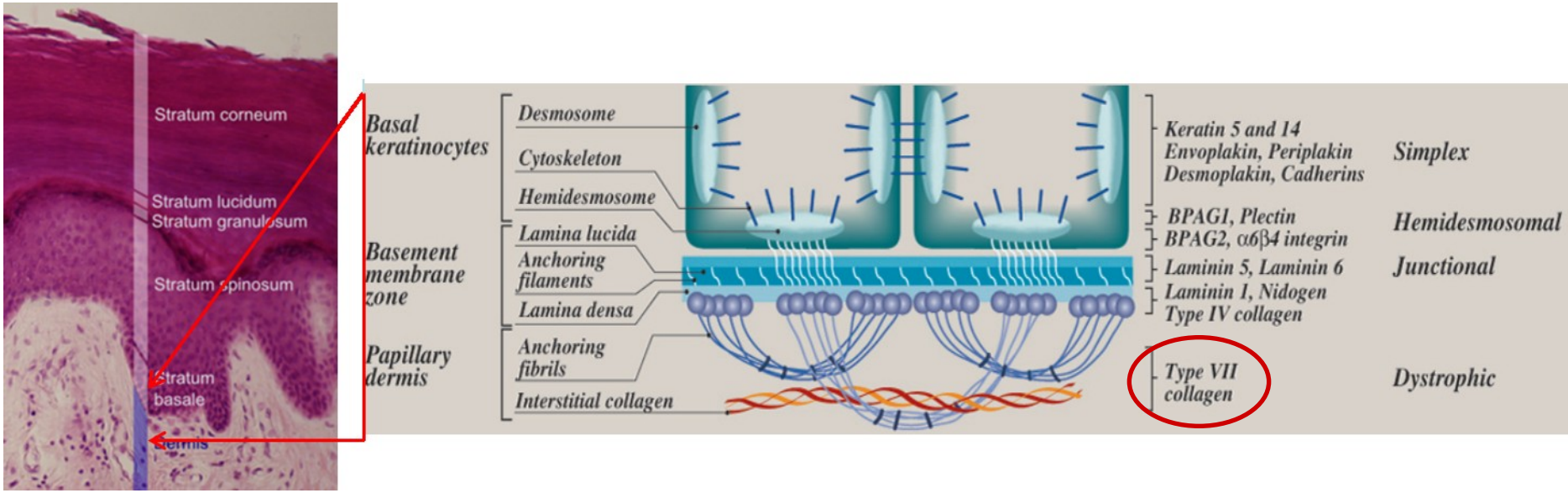
(*FNuSA, PAU*)

4. DNA nalýza (PCR-sekvenční analýza)

(*FNB, CMBGT*)



Dystrophic epidermolysis bullosa (DEB)

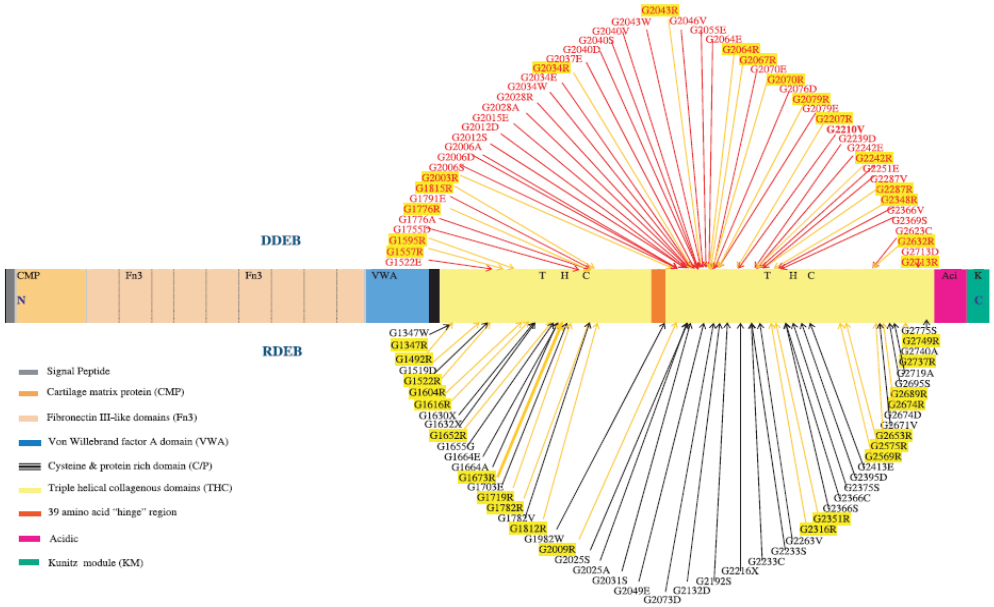


- **The *COL7A1* gene** (3p21, 118 exons); procollagen VII alpha chain.
- **DEB** is inherited in both **autosomal dominant** (DDEB) and **autosomal recessive** manner (RDEB).

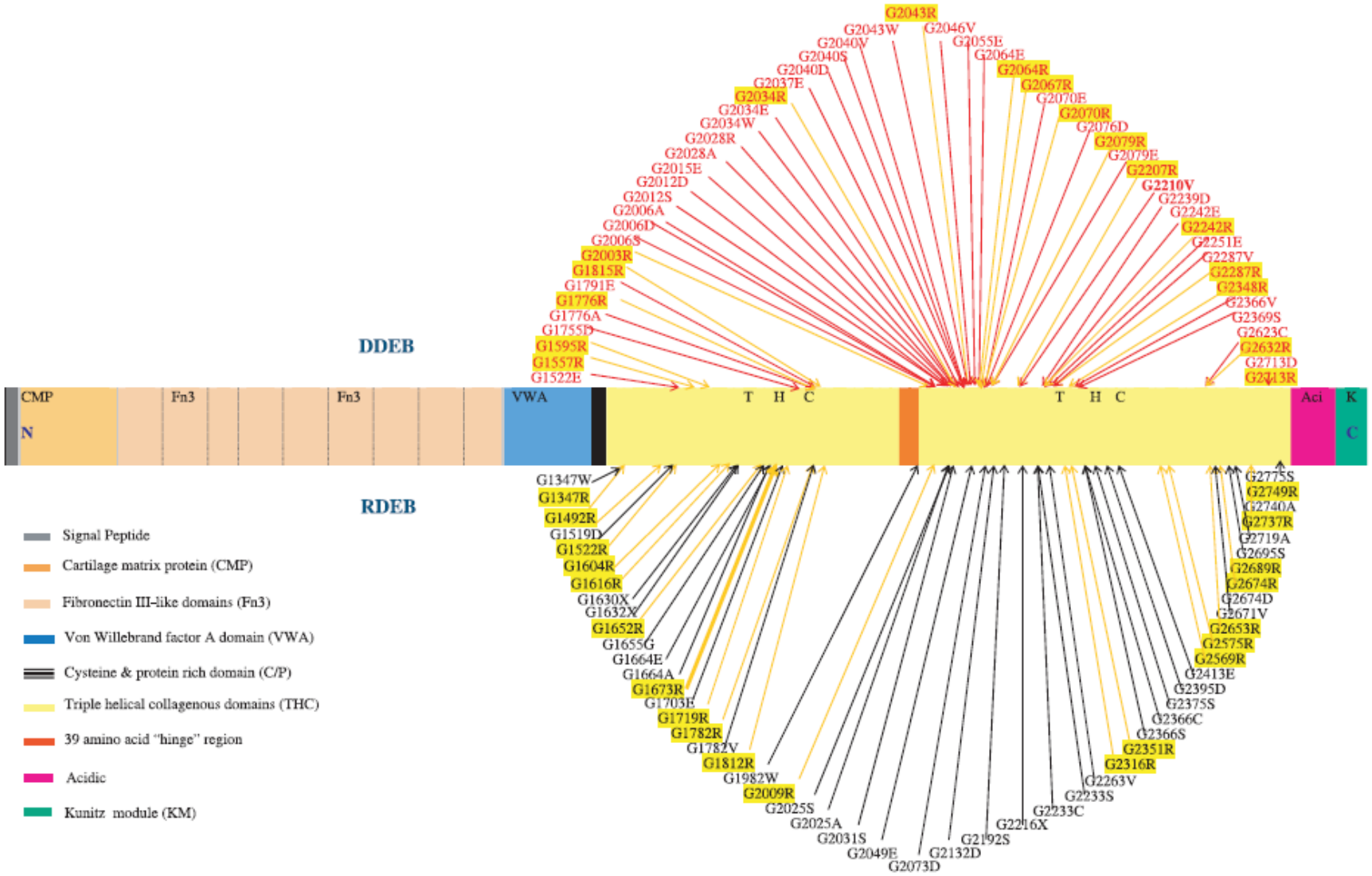
The clinical features of DEB have a broad range of severity:

- **The <u>severe</u> RDEB,** associated with **premature termination codon (PTC) mutations** on both *COL7A1* alleles.
- **The <u>milder</u> RDEB,** caused by **compound heterozygous mutations: one PTC mutation and one missense mutation** (frequently glycine substitutions).

- **DDEB usually involves glycine substitutions** within the triple helical domain of COL7A1.

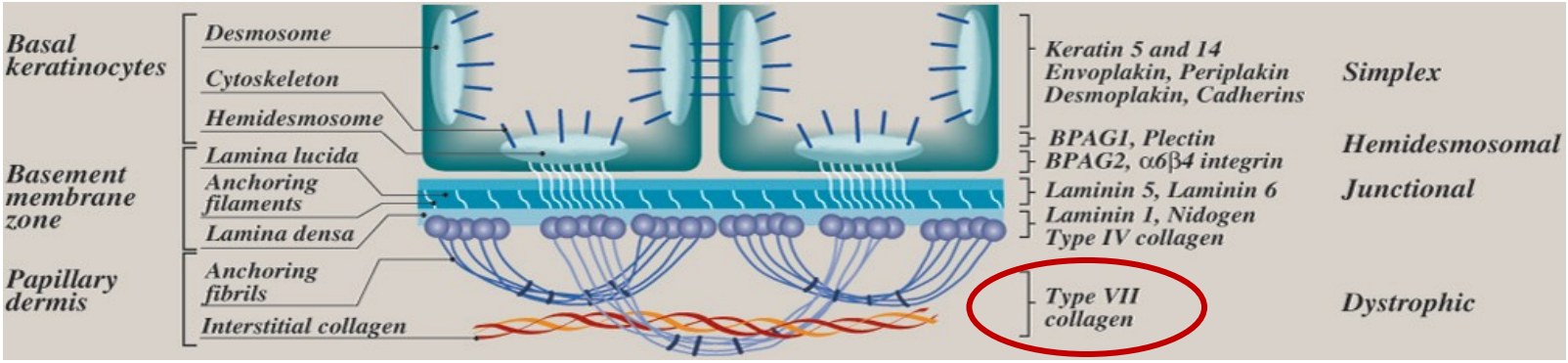


COL7A1 missense and nonsense mutations in DEB patients. The red lettering signifies dominant and the black signifies recessive.
N. Dang 2008

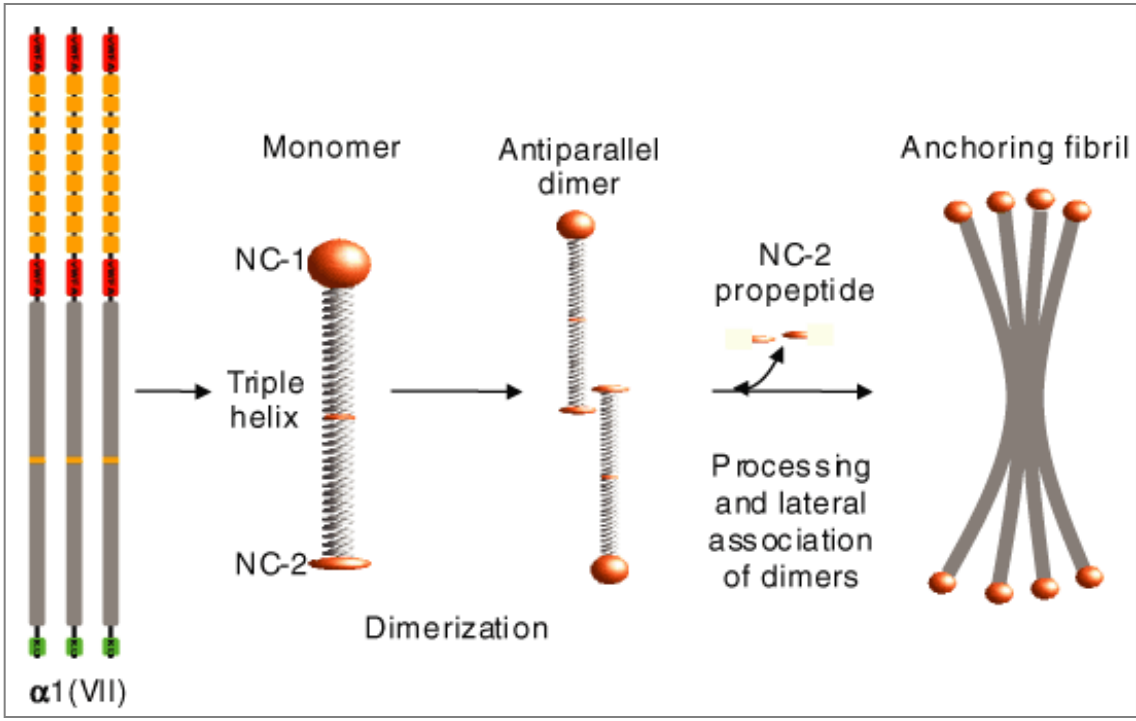


COL7A1 missense and nonsense mutations in DEB patients. The red lettering signifies dominant and the blue signifies recessive. *N. Dang 2008*

Dystrophic epidermolysis bullosa (DEB)



- *COL7A1* - **procollagen VII alpha 1 chain**. Each proa1(VII) chain contains a central triple helical collagenous domain flanked by amino-terminal (NC-1) and carboxy-terminal (NC-2) non-collagenous domains.
- The triple helical domain consists of a repeating **Gly-X-Y sequence**.
- Three proa1(VII) chains folded into monomer. Two monomers form an antiparallel dimer, from which the NC-2 propeptides are removed proteolytically. Finally, the mature dimers laterally aggregate into anchoring fibrils.

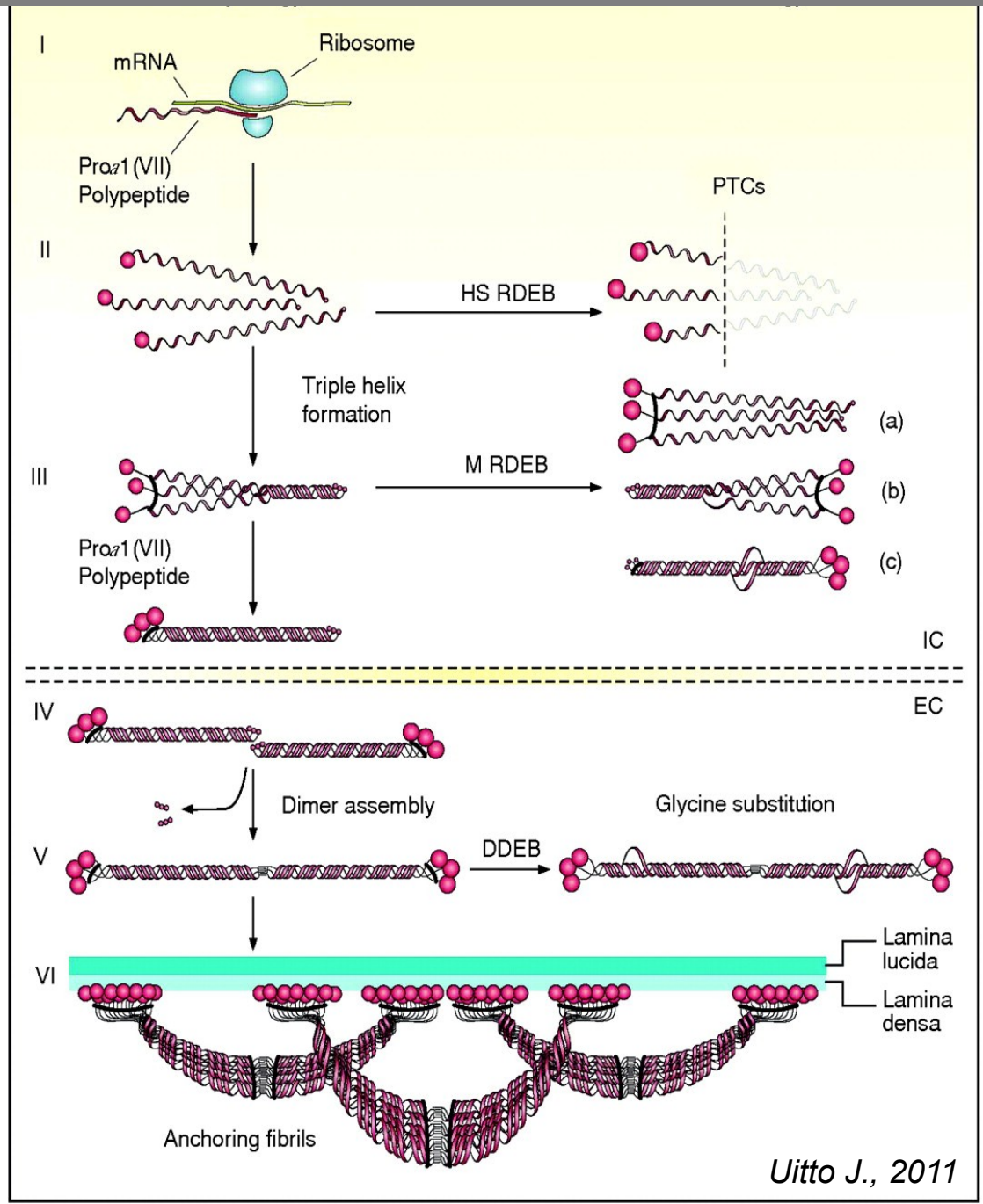


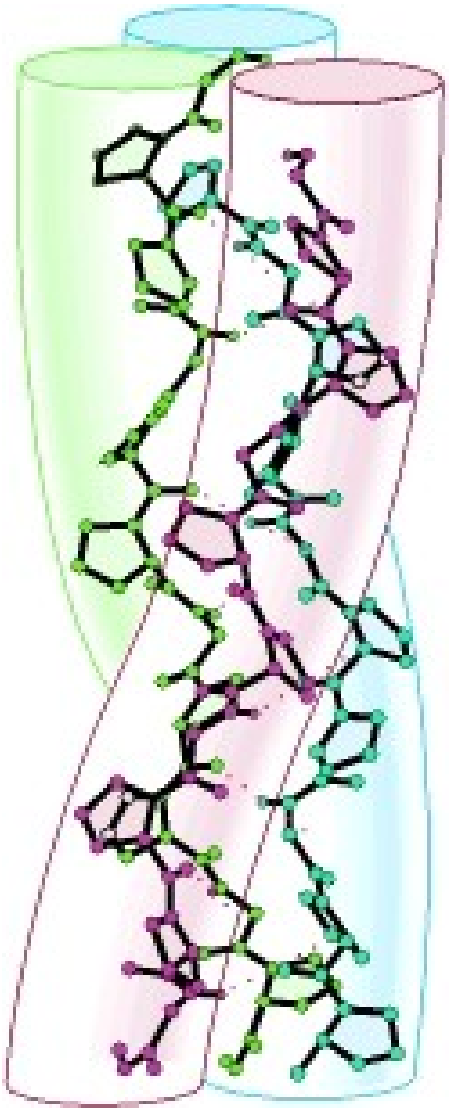
Anchoring fibril assembly.

- I: proa1(VII) are synthesized.
- II, III: three chains assemble into the type VII collagen molecule.
- IV, V: two type VII collagen molecules form the antiparallel dimer, the NC-2 domains are removed, and association of the monomers is stabilized by intermolecular disulphide bonds.
- VI: a large number of dimer molecules assemble into anchoring fibrils and the NC-1 domain keeps the adhesive property at both ends.

PTC mutation are associated with nonsense mediated mRNA decay (NMD). Missense mutations alter homotrimer formation.

Glycine substitutions often happen in triple helix region of COL7A1 affecting correct folding and secretion of the type VII collagen.

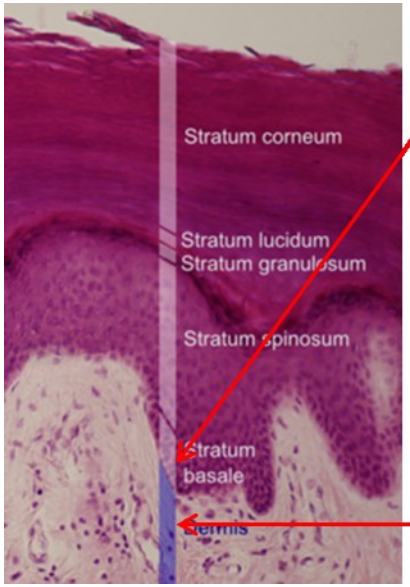


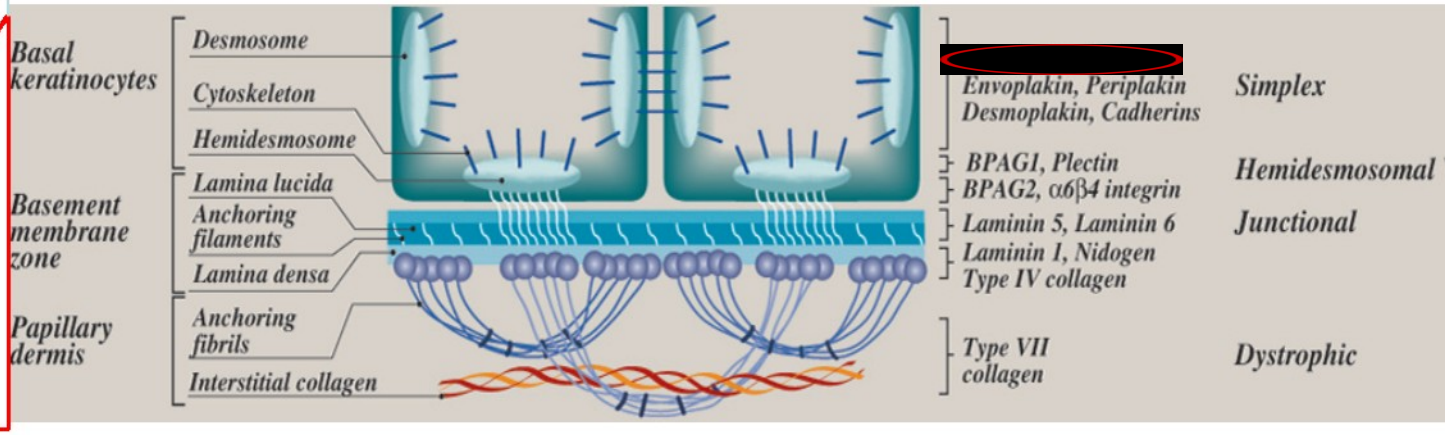


Structure of collagen

- Three procollagen VII alpha 1 chains coil around one another in characteristic triple helix structure.
- The AA sequence of collagen triple helix domain consists of **Gly-X-Y repeats**.
- **The strict preservation of Gly in every third position is required for close packing of three supercoiled helices that make up the collagen triple helix structure. Gly residues are buried at the center of the triple helix, in a position that accommodates no other residue.**
- In contrast, the X and Y positions are exposed on the surface. The X position is frequently occupied by proline and the Y position by hydroxyproline.

Epidermolysis bullosa simplex

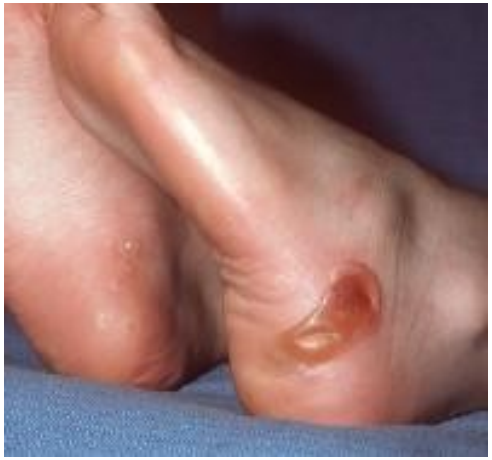


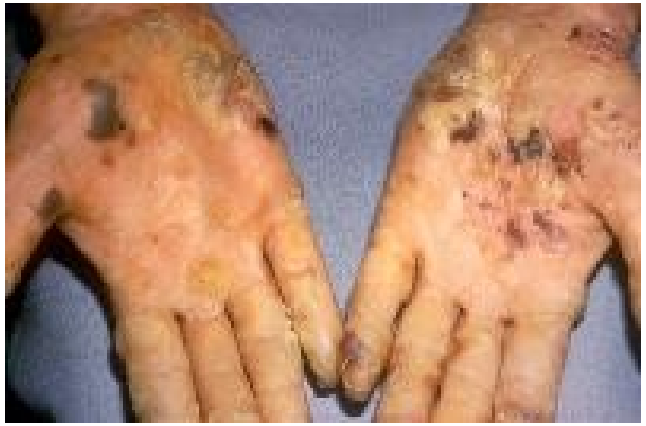


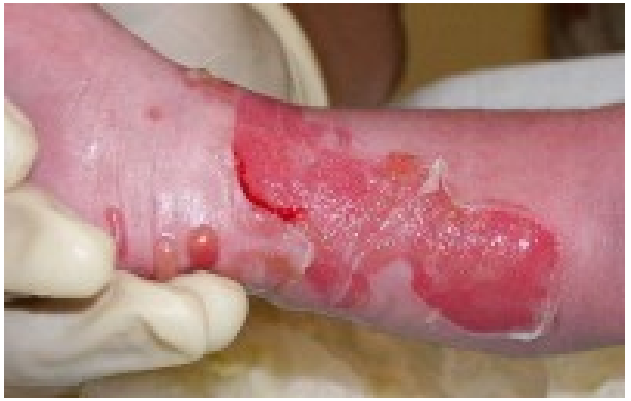
Three main autosomal dominant subtypes of EBS are distinguished based on the severity of blistering:

- **EBS localized** - blistering is usually limited to the hands and feet.
- **EBS another generalized** - more widespread blistering is observed but it is usually milder than in the most severe variant.
- **EBS Dowling-Meara** - the most severe variant, characterized by generalized herpetiform blistering especially in the neonatal and infant periods.

- These subtypes of EBS are caused by mutations in either **the keratin 5** (*KRT5*) or **the keratin 14** (*KRT14*) genes. **Most keratin mutations are inherited in an autosomal dominant manner.**

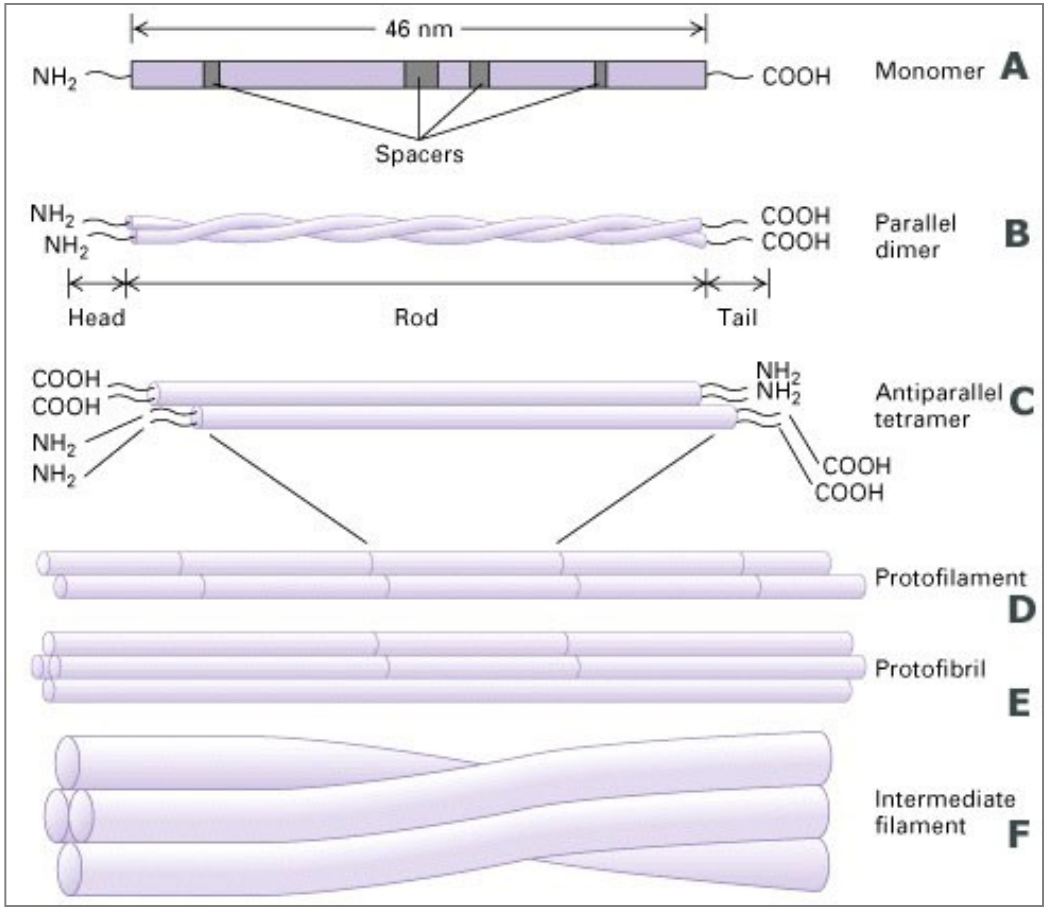






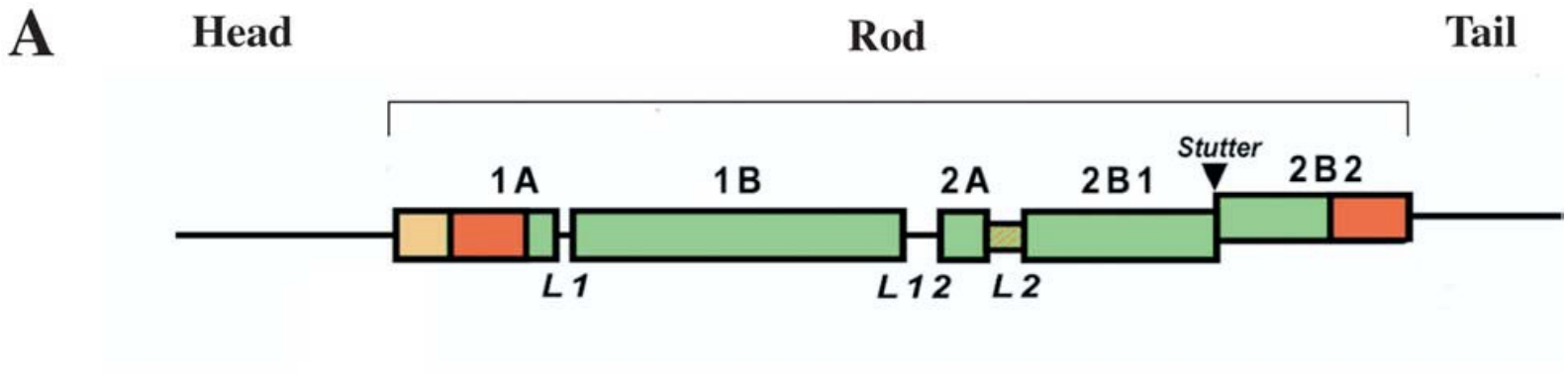
• Keratins are a group of structural proteins that polymerize to form **keratin intermediate filaments.**

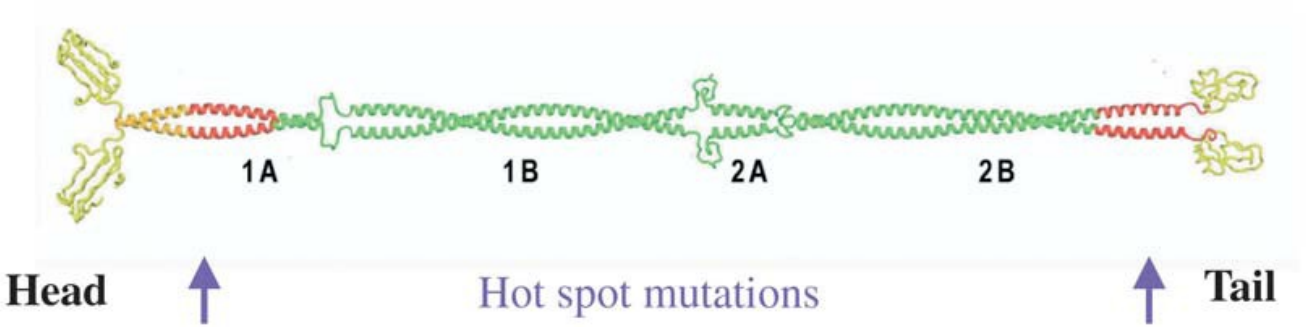
• A characteristic feature of all intermediate filament proteins is the central alpha-helical rod domain, which is divided into four helices (1A, 1B, 2A and 2B) by three short nonhelical linker domains and terminated by globular head domains.

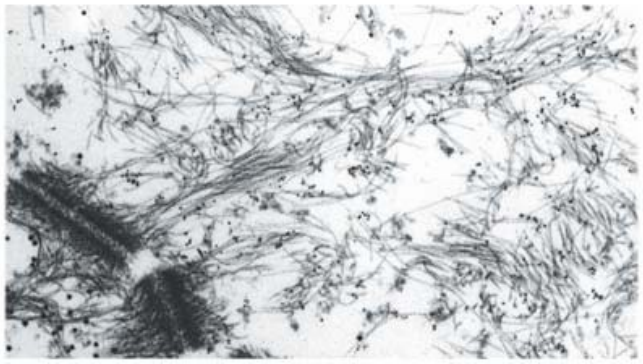


• Keratins are divided into type I (KRT9–KRT24) or type II (KRT1–KRT8) proteins according to their physical and chemical properties, and the basic structural unit of intermediate filaments is a heterodimer of one type I keratin and its corresponding type II partner.

• **Keratins 5 and 14 are natural partners that dimerize by coiled-coil interactions.**



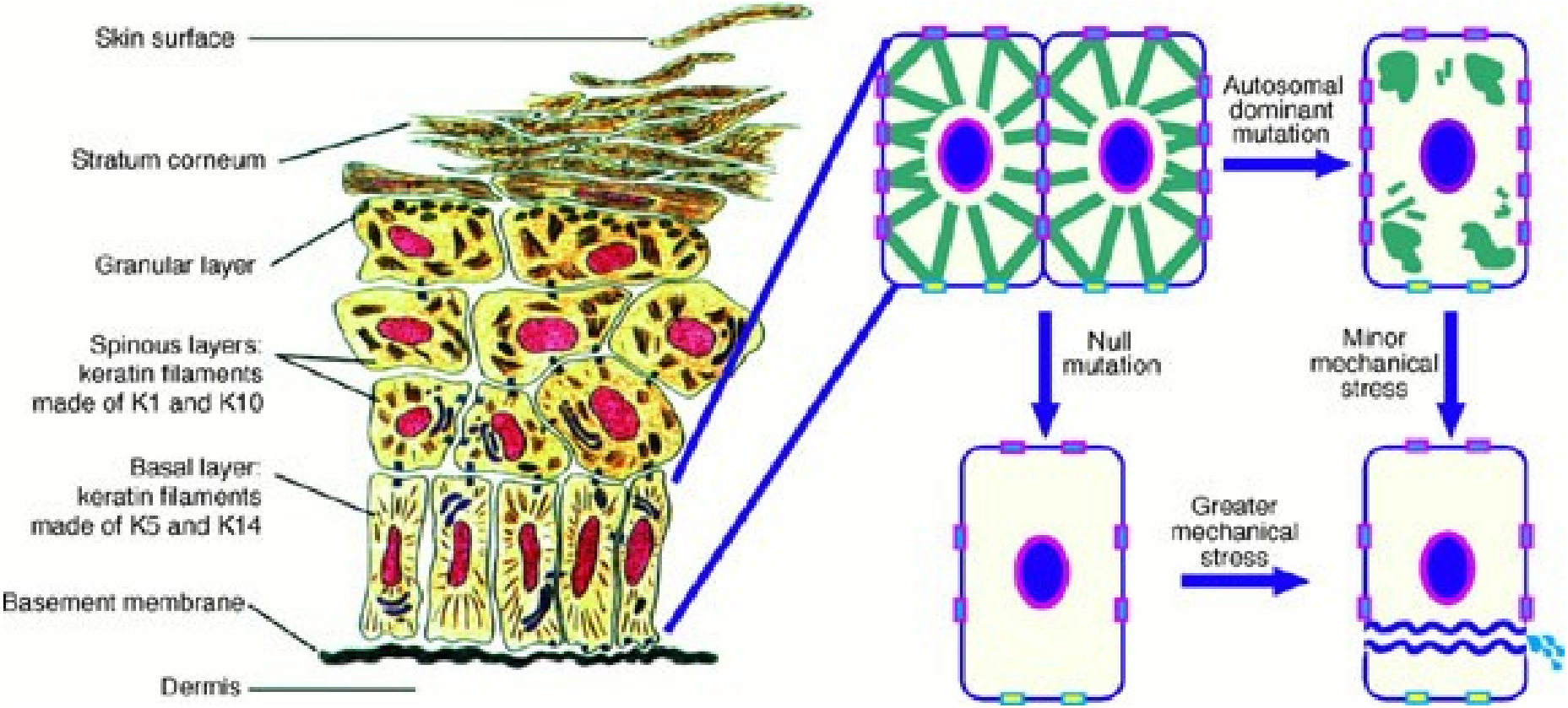




1A, 1B, 2A, 2B: a-helical parts; L1, L12, L2: non-*α*-helical parts, also called 'linkers'. The *α*-helical segments exhibit a heptad substructure (abcdefg), where the a and d positions are occupied by apolar AAs. These hydrophobic AAs generate a surface that is wound around the axis of a single right-handed *α*-helix in a left-handed manner, ultimately leading to superhelix, i. e. coiled-coil formation of two such molecules. The phasing of the heptads is broken in the middle of segment 2B giving rise to a 'stutter'. The stutter represents a helical segment which is not engaged in coiled-coil formation. The formation of heterodimers in which type I and type II polypeptide chains align in parallel and in exact axial register is the first step of keratin filament assembly. Two heterodimers associate, forming tetramer units aligned in an antiparallel manner.

Kirfek J., Cell. Mol. Life Sci. 60 (2003)

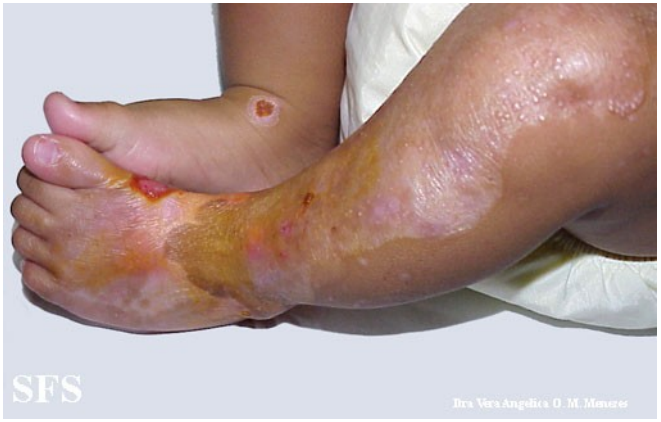
Epidermolysis bullosa simplex (EBS)

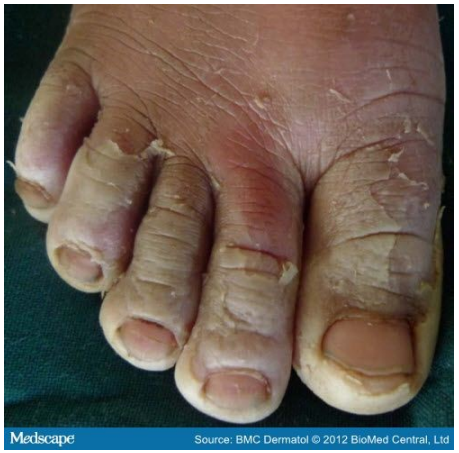


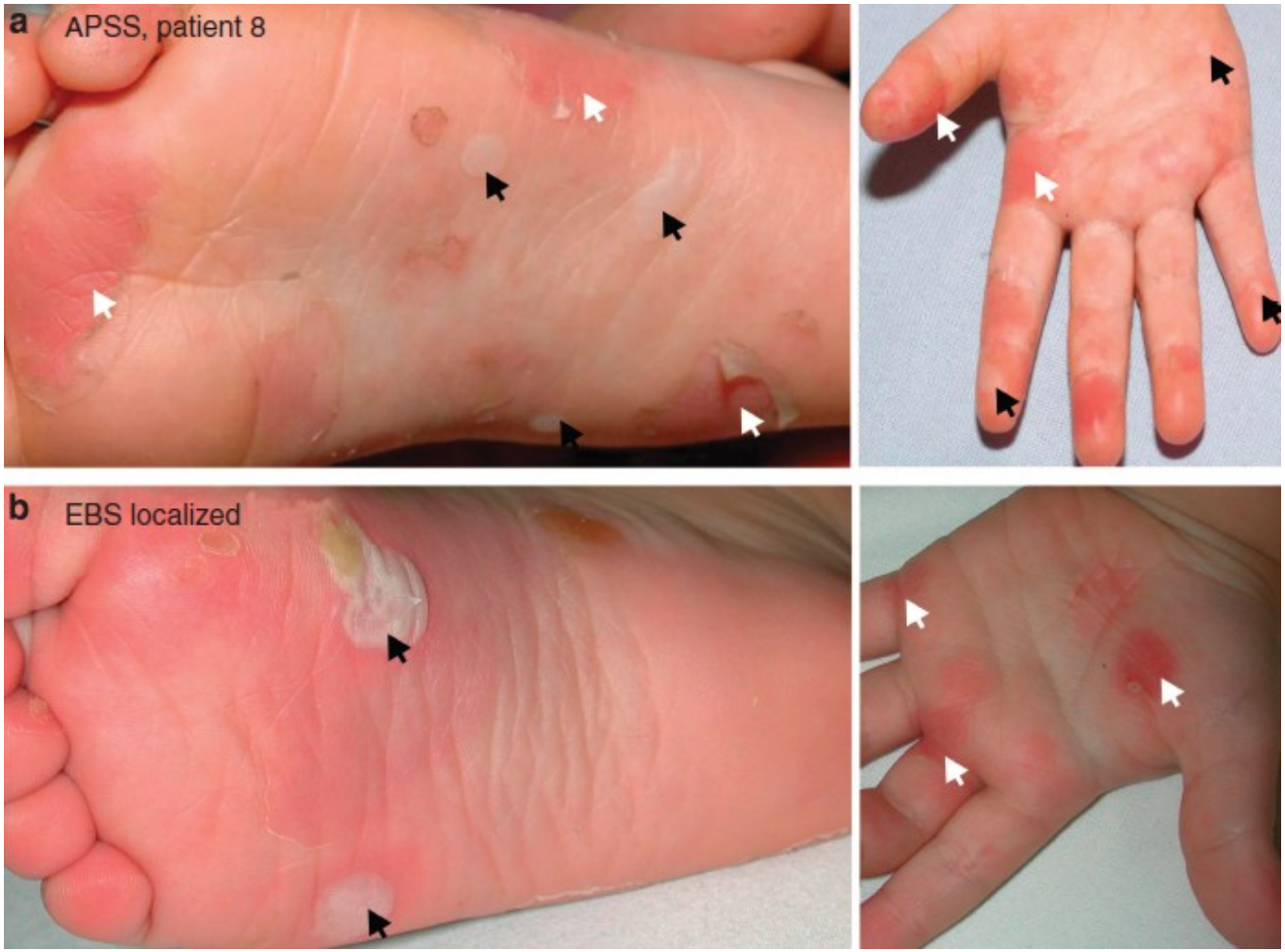
The image illustrates the cell in EBS patients. It displays what a normal cell cytoskeleton ought to look like and what happens in EB disease states.

From 75 probands with EBS:

- **26 EBS (34.7%), a mutation in *KRT5* or *KRT14*, AD**
  - the rate of *de novo KRT5* and *KRT14* mutations is 32.0%
- **40 superficial EBS/acral peeling skin sy., mutations in *TGM5* (53.3%), AR**
- **1 EBS with muscular dystrophy, mutations in *PLEC* (1.3%), AR**
- 8 without a detectable causal mutation (10.7%)







***TGM5* – AR – APSS**

***KRT14* – AD – EBS**

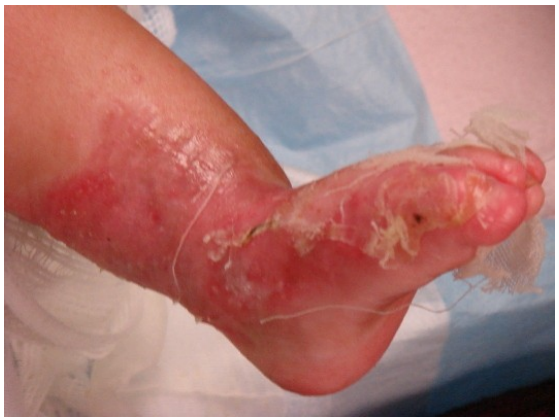
D. Kiritski 2010

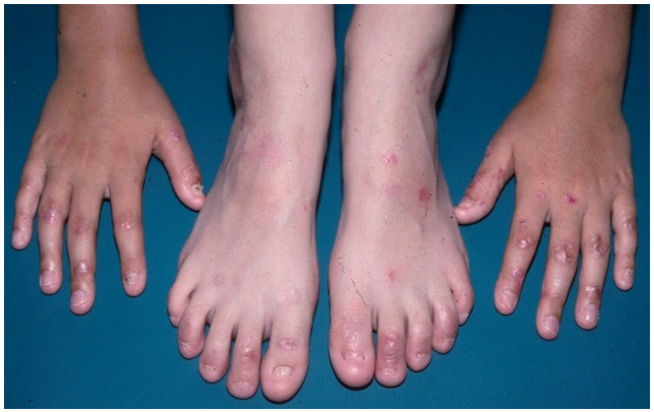
Similar clinical features in the acral peeling skin syndrome (APSS) and localized EBS in young children. ***(a) Blisters (black arrows) and erosions (white arrows) on the palms and soles of the 2-year-old patient with APSS, and (b) of a 2-year-old patient with localized EBS heterozygous for the keratin 14 mutation.***

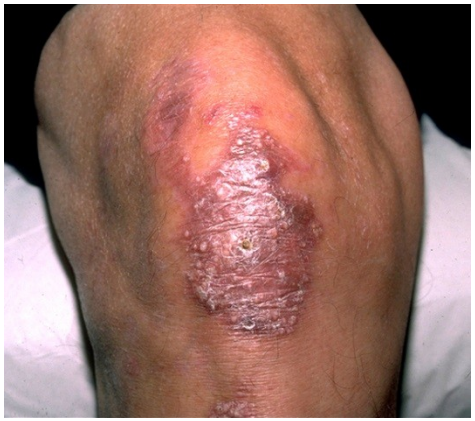
From 61 probands with DEB:

- **21, dominant DEB (34.4%), a mutation in *COL7A1***
  - the rate of de novo *COL7A1* mutations is 21.1%
- **39, recessive DEB (63.9%), mutations in *COL7A1***
- 1 with recessive DEB, only one mutation identified (1.6%).

At present, we have 57 living DEB patients in the Czech Republic
⇒ **the estimated prevalence of DEB 5.4 cases per million inhabitants in the overall Czech population.**



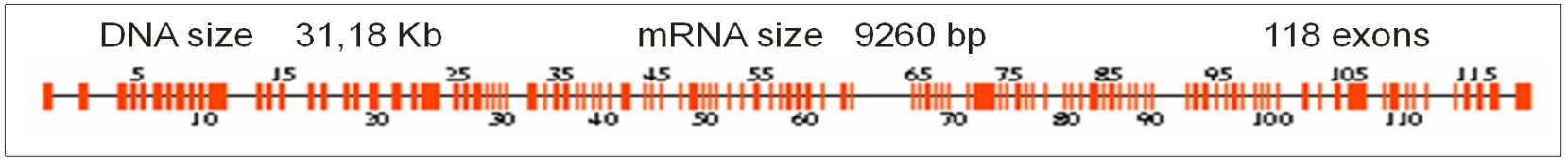




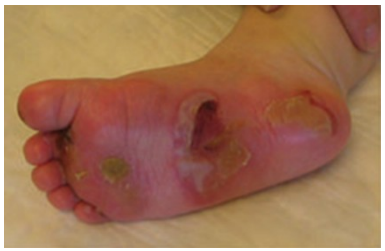
Genodermatoses are a large group of clinically and genetically heterogeneous disorders.

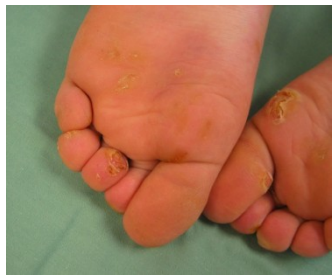
2005: DNA diagnostics of epidermolysis bullosa (EB)

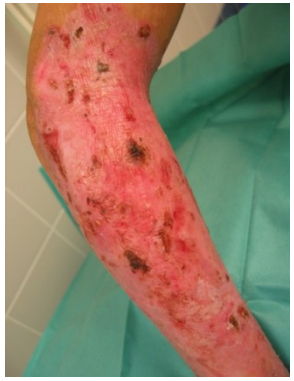
- **Dystrophic EB** - PCR and direct sequencing of *COL7A1*
- **Simplex EB** - PCR and direct sequencing of *KRT5* and *KRT14*

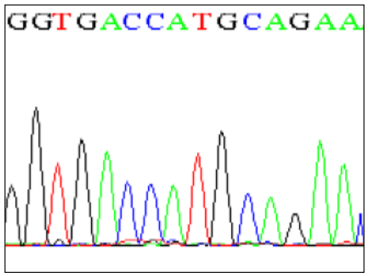


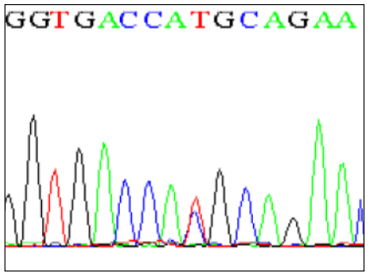
COL7A1 gene: distribution of exons and introns







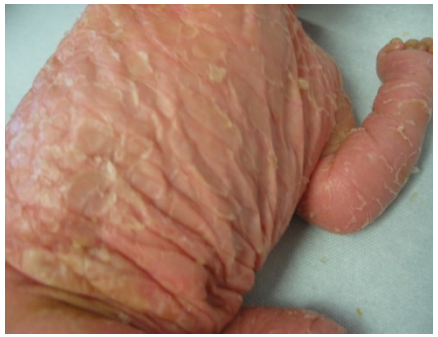


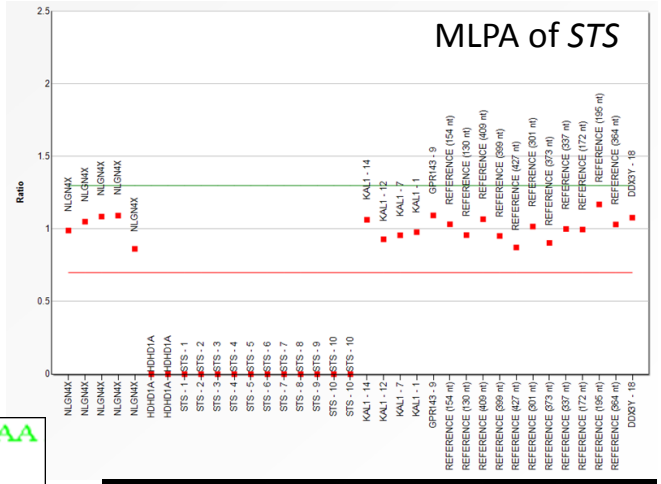


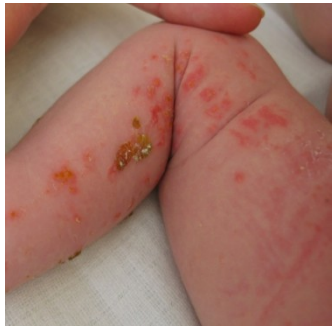
2012: DNA diagnostics of ichthyoses

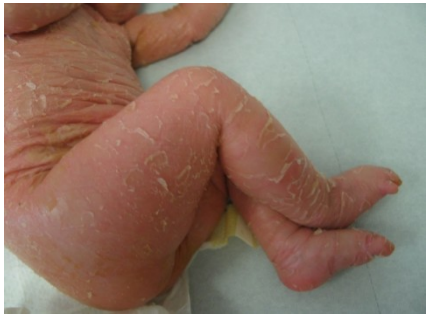
- **Autosomal recesive congenital ichthyoses** – PCR and direct sequencing of *TGM1, ALOX12B, ALOXE3, CYP4F22, NIPAL4*
- **Recesive X-linked ichthyosis –** MLPA and direct sequencing of *STS*

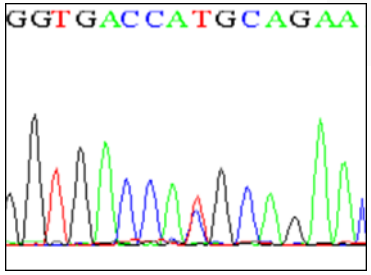
2012: DNA diagnostics of incontinentia pigmenti – PCR and fragment analysis, PCR and direct sequencing of *NEMO/IKBKG*

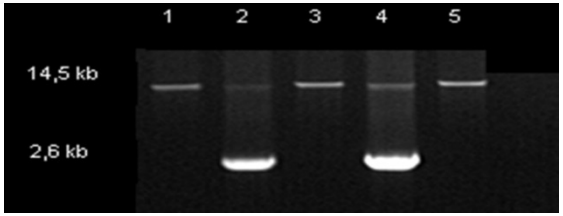








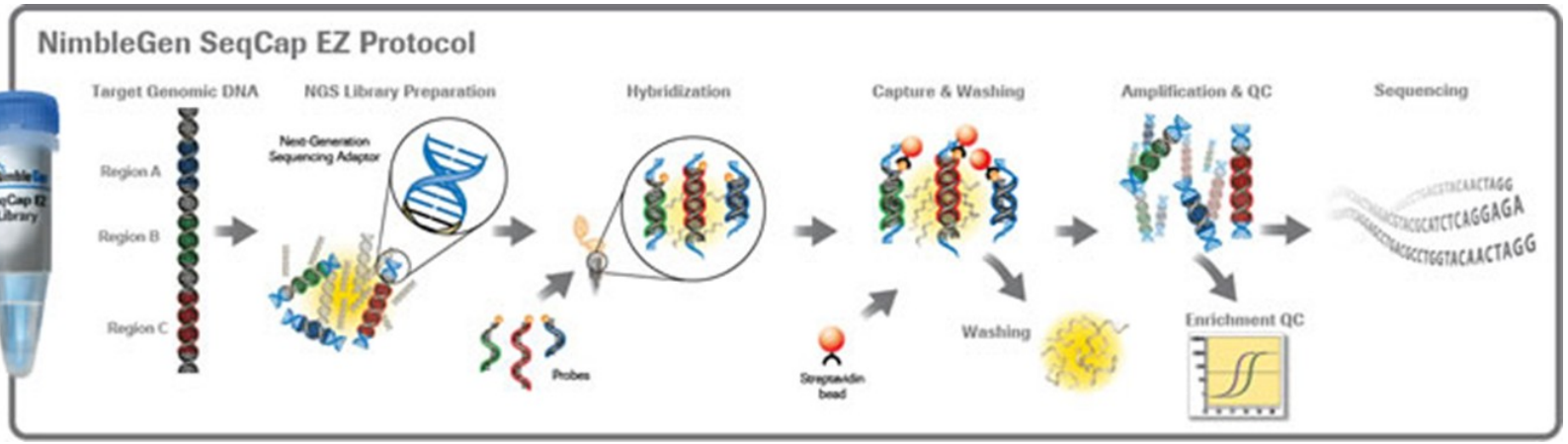




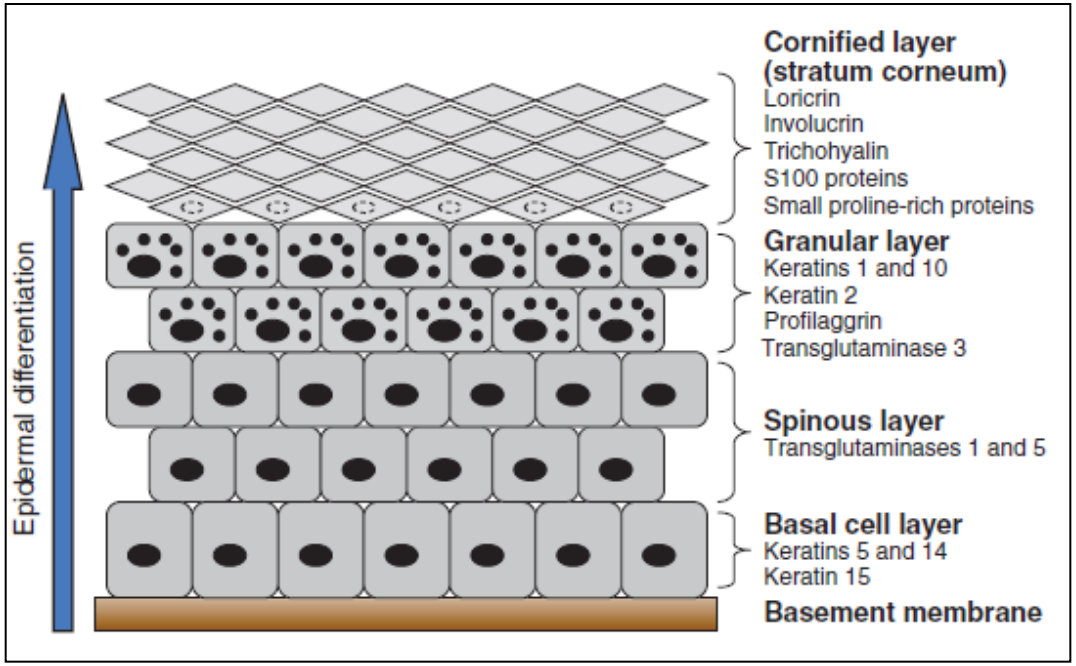
PCR-fragment analysis of *NEMO*

2014

- „Sequence Capture“ and targeted sequencing of 100 genes associated with genodermatoses

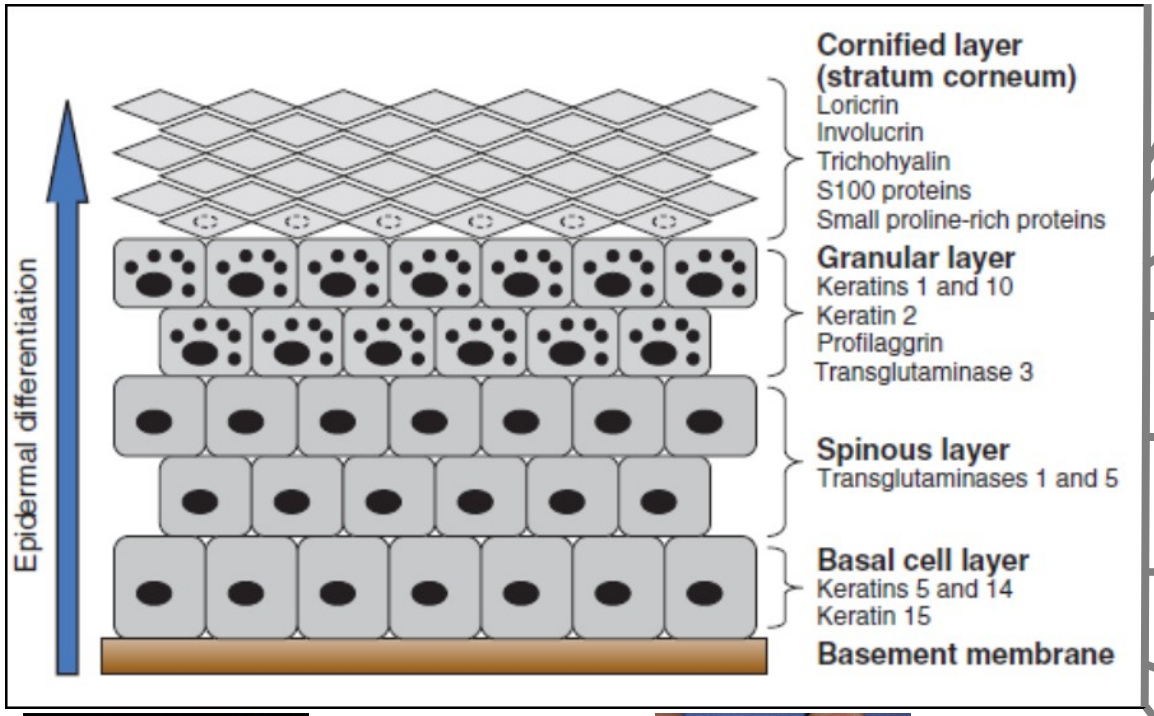


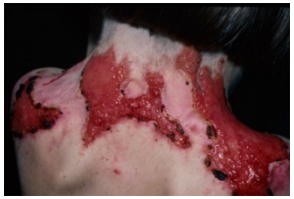
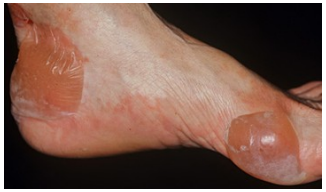
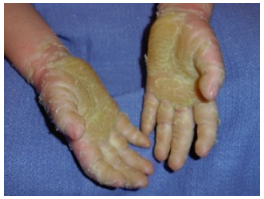
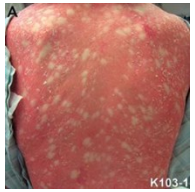
- Epidermis is separated from dermis by the basement membrane.
- **Keratinocytes**, which compose epidermis, proliferate within the basal cell layer. Basal keratinocytes express **KRT5, KRT14 and KRT15.**
- As basal keratinocytes commit to **terminal differentiation**, they switch off the expression of KRT5, KRT14 and KRT15 and induce the expression of **KRT1, KRT10 and KRT2**.
- **As differentiation proceeds, keratinocytes progress upwards through the different epidermal layers becoming anucleated and increasingly compacted in size, before being lost from the skin surface by desquamation.**



A. Sandilands, Journal of Cell Science 2009

- **Epidermolysis bullosa –** clinically and genetically heterogeneous group of disorders characterised by fragility of the skin and blistering.
- **Ichthyoses –** clinically and genetically heterogeneous group of disorders characterized by dry, thickened, scaly or flaky skin; in many types there is cracked skin, which is said to resemble the scales on a fish.



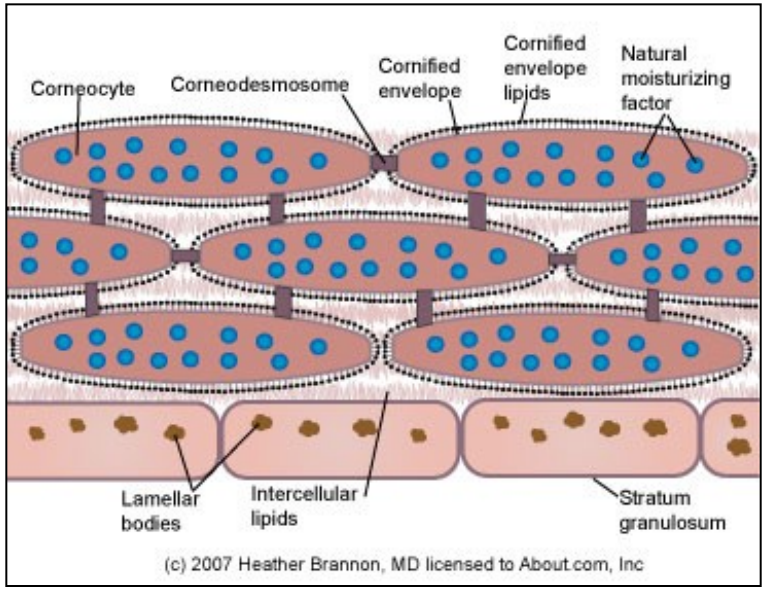





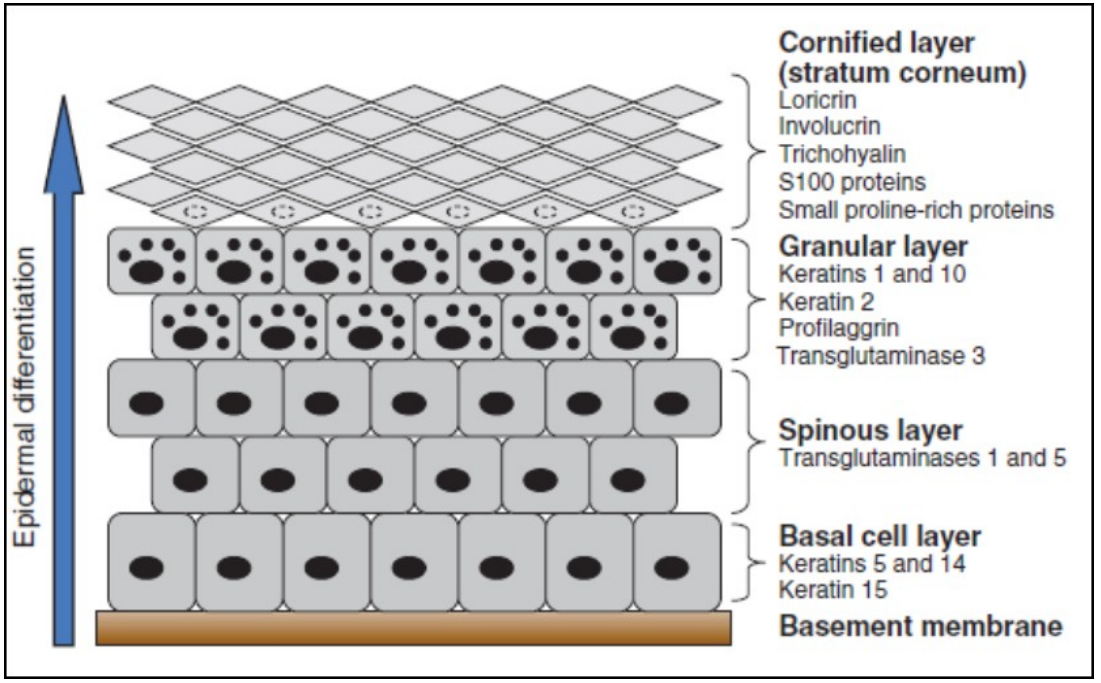
- RXLI (*STS*)
- Ichthyosis vulgaris (*FLG*)
- ARCI (*TGM1, ABCA12, NIPAL4, CYP4F22, ALOX12B, ALOXE3, PNPLA1, LIPN, CERS3*)
- Keratinopatic ichthyoses (*KRT1, KRT2, KRT10*)
- EB superficialis (*TGM5*)
- EB simplex (*KRT5, KRT14, DSP, DST, JUP, PKP1, EXPH5, PLEC*)
- EB junctional (*COL17A1, LAMA3, LAMB3, LAMC2, ITGA6, ITGB4, ITGA3*)
- EB dystrophic (*COL7A1*)

Clinically and genetically heterogeneous group of disorders characterized by scaling of the skin.

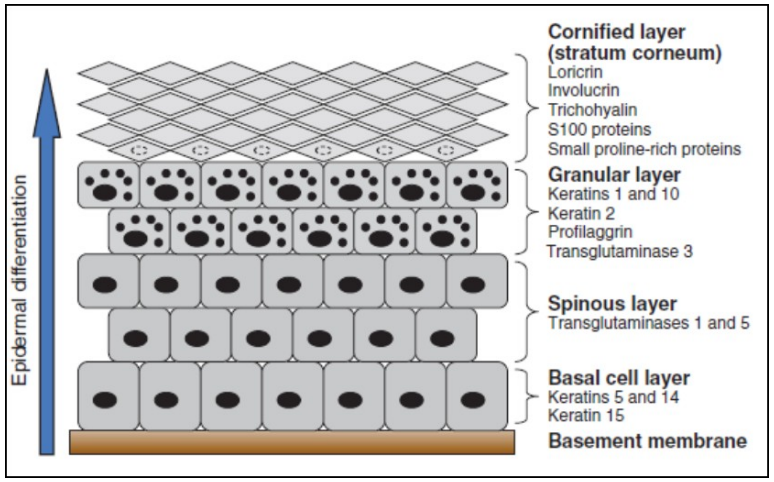
- **Common ichthyoses** (ichthyosis vulgaris - *FLG*, autosomal semidominant; recessive X-linked ichthyosis - *STS*)
- **Keratinopathic ichthyoses** (*KRT1, KRT10, KRT2*) – autosomal dominant
- **Autosomal recessive congenital ichthyoses** (*TGM1, ABCA12, NIPAL4, CYP4F22, ALOX12B, ALOXE3, PNPLA1, LIPN, CERS3*)

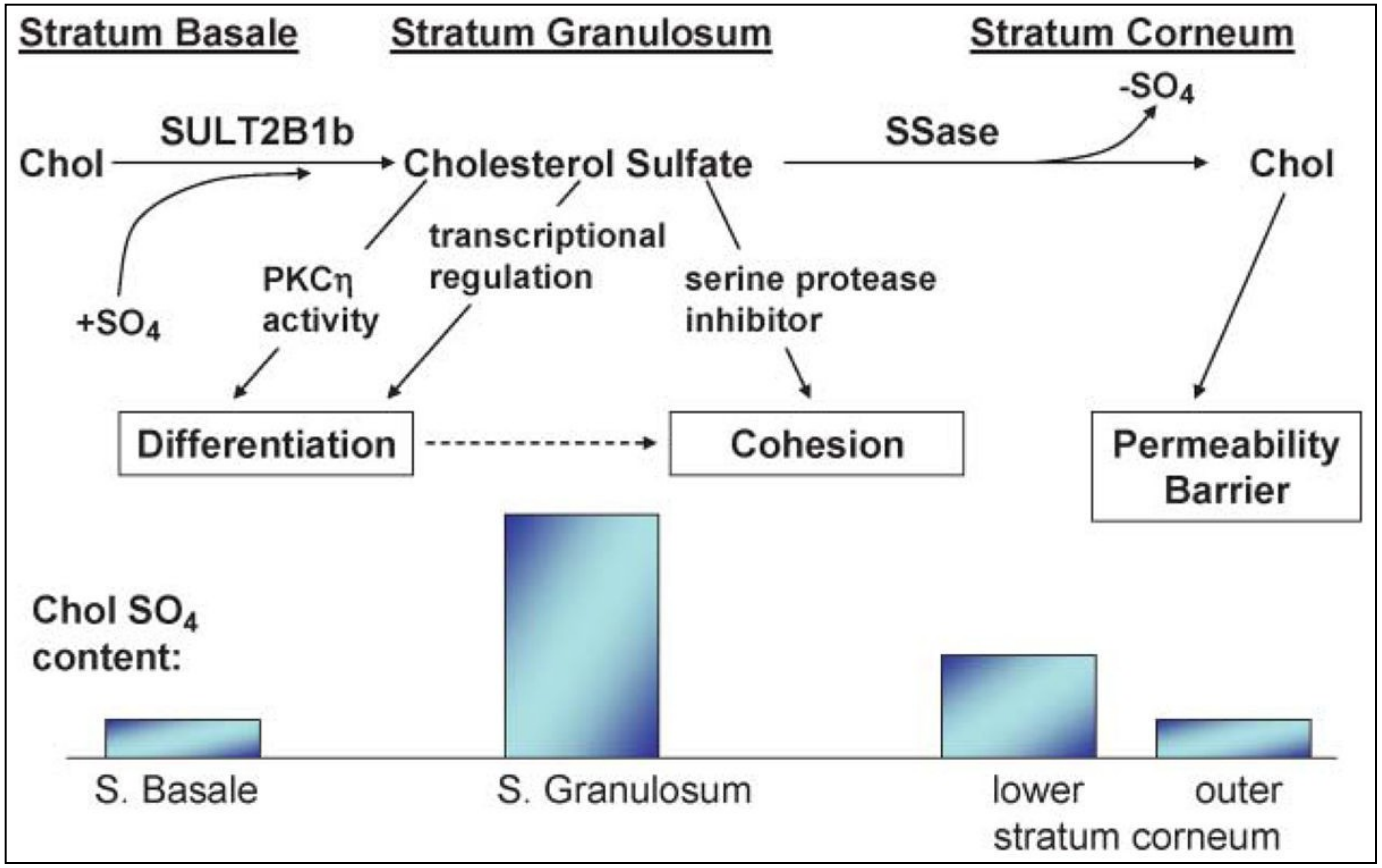
- **affects 1 in 2000 - 6000 males**
- **caused by deficiency of the steroid sulfatase enzyme (a mutation in the *STS* gene)**
- STS is secreted into intercellular space of stratum corneum.
- STS degrades cholesterol sulphate, generating cholesterol. The progressive decline in cholesterol sulphate permits corneodesmosome degradation leading to normal desquamation.

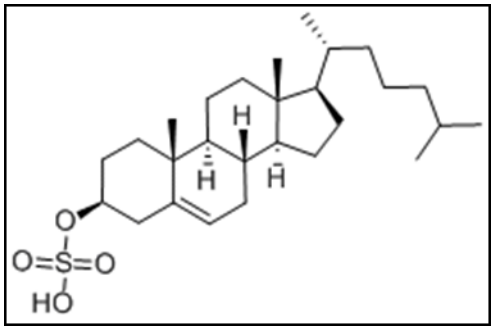


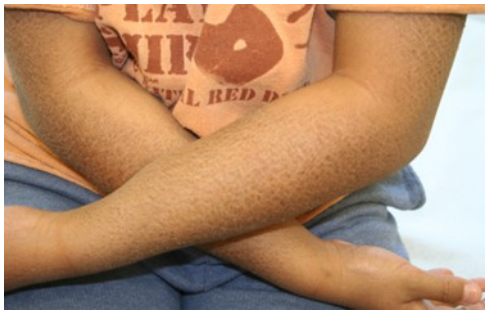


- Cholesterol sulfotransferase (SULT2B1b) generates cholesterol sulfate in s. granulosum.
- Steroid sulphatase (Ssase, STS) desulfated cholesterol sulfate back to cholesterol in s. corneum.
- Disruption of this cycle accounts for abnormal desquamation and disruption of the skin barrier function.





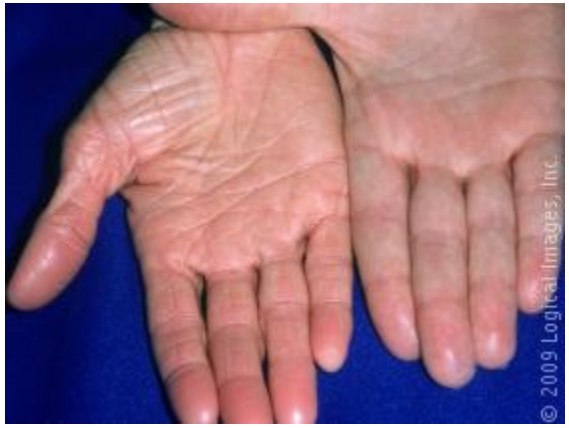


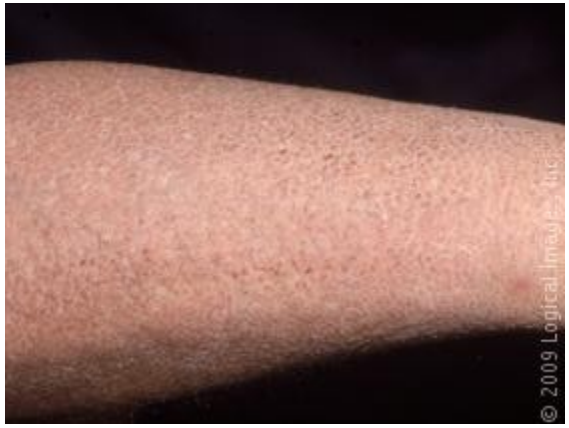


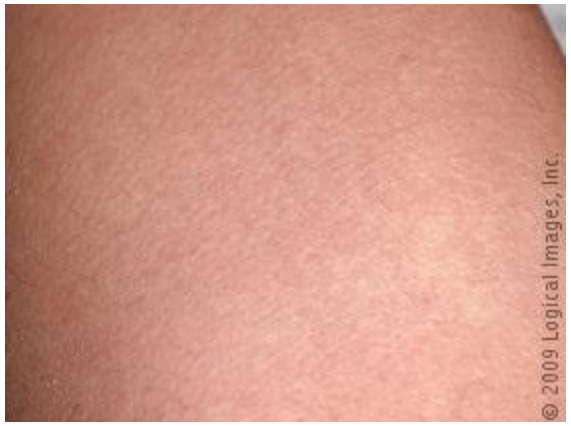
P.M. Elias, J Lipid Res. 2008

- **the most common form of ichthyosis, affecting 1 in 250 people**
- **associated with null mutations (mutations creating premature termination codon) in the *FLG* gene**
- **autosomal semidominant inheritancce with incomplete penetrance and variable expressivity**

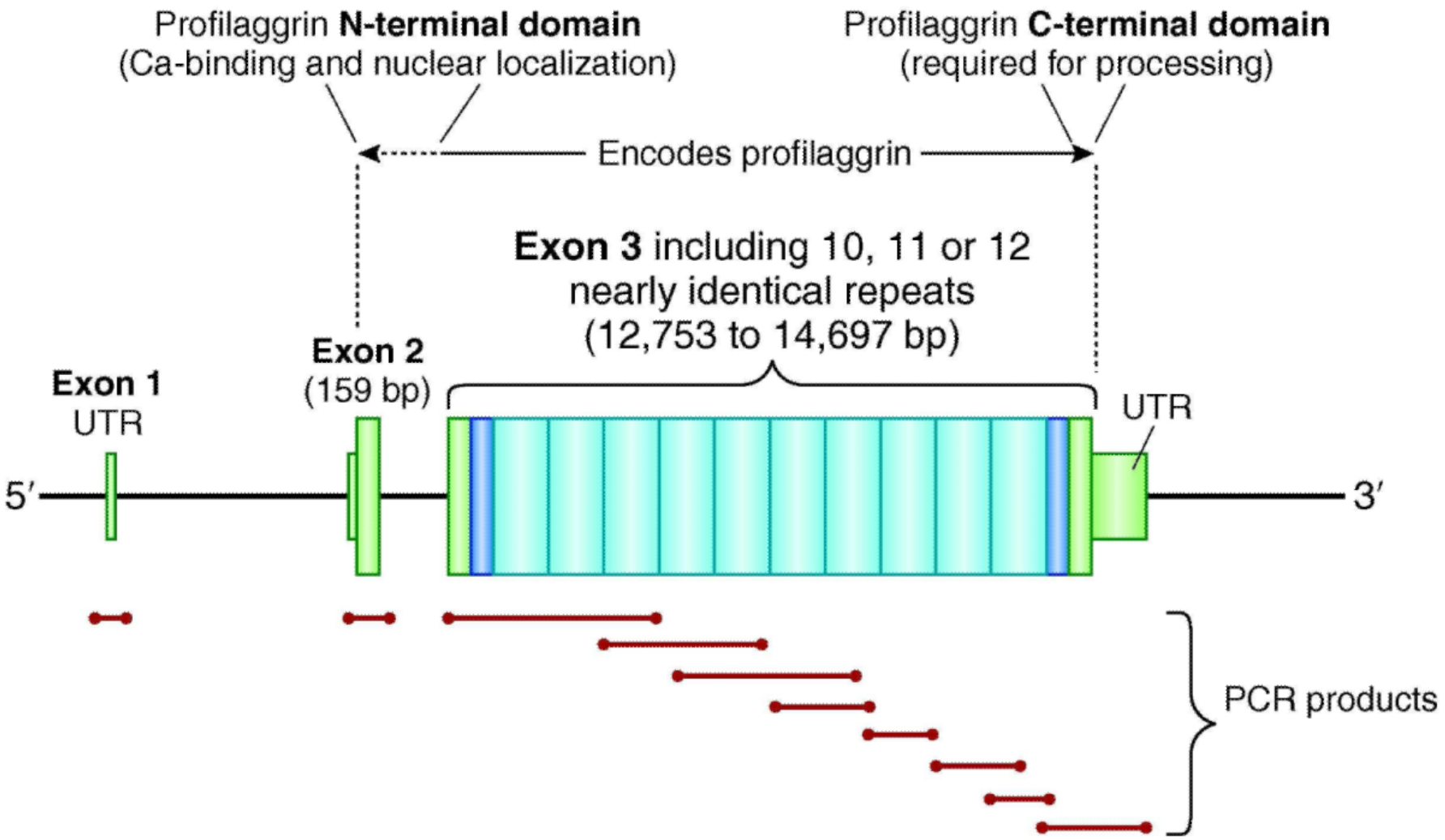
- disease severity can vary considerably even within affected families
- IV can be asymptomatic, although patients may complain of dry, rough skin and cosmetic embarrassment
- individuals with two *FLG* null mutations have a more severe phenotype than those with one *FLG* null mutation.





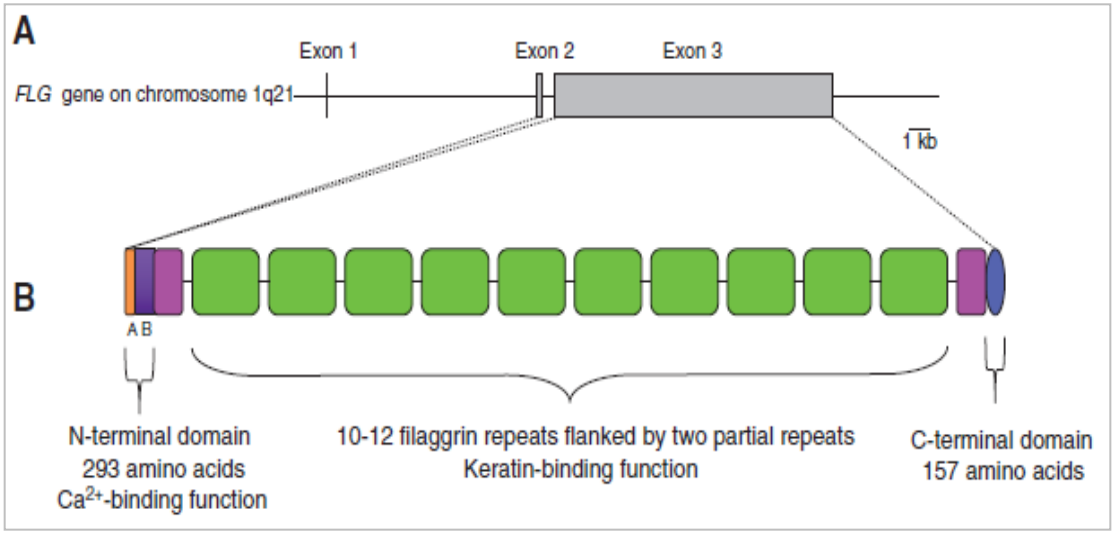


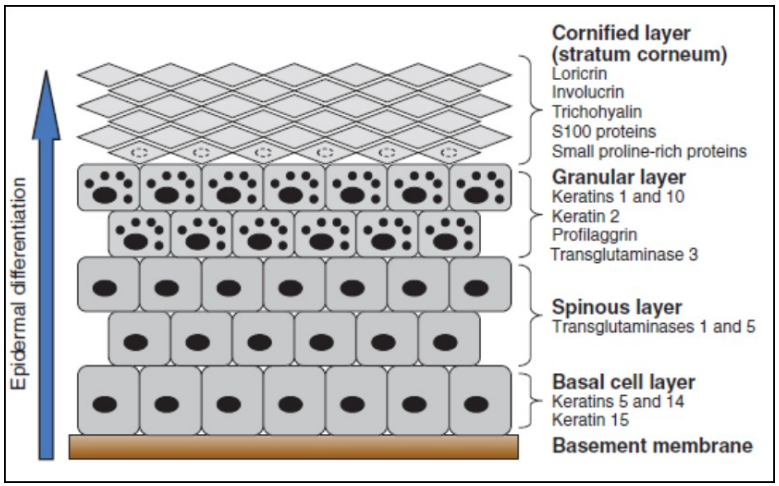
The *FLG* gene structure



Diagrammatic representation of the *FLG* gene structure

S. J. Brown, J Invest Dermatol. 2012



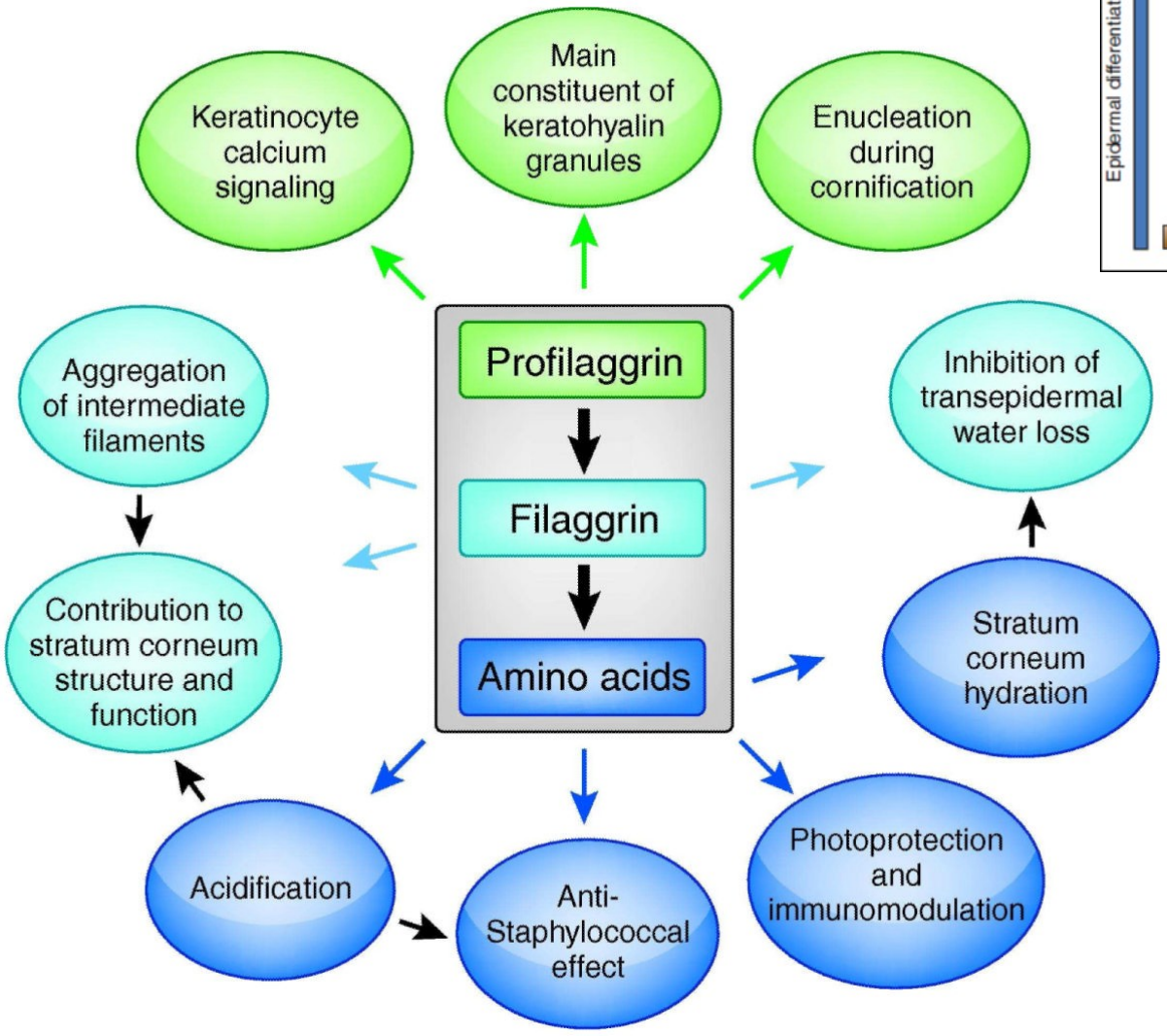


A. Sandilands, Journal of Cell Science 2009

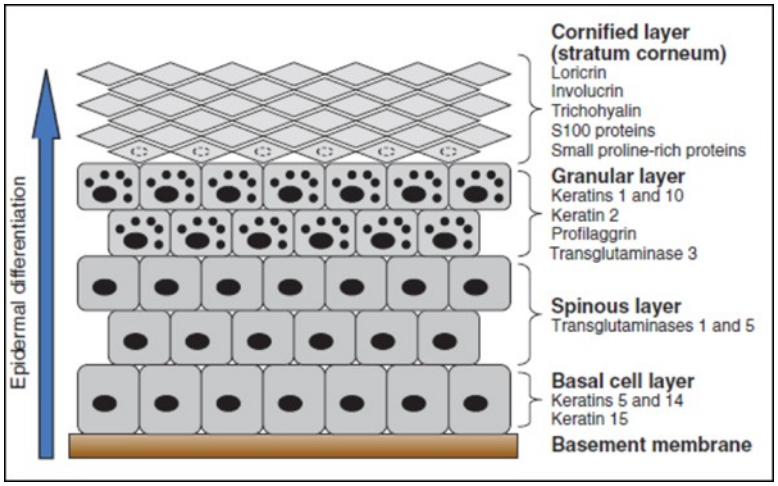
- **The profilaggrin N-terminal domain plays role during terminal epidermal differentiation** - the N-terminal domain is cleaved and translocates to the nucleus where it plays role in enucleation of keratinocytes in stratum corneum.
- The precise function of **the profilaggrin C-terminal domain** is unclear but it is known to be required for the processing of profilaggrin to filaggrin.
- 10, 11 or 12 nearly identical filaggrin repeats (324 AA) have keratin binding properties. The large (>400kDa) insoluble profilaggrin is degraded to produce monomeric filaggrin in stratum corneum and then further proteolyzed to release amino acids.

Keratin-filaggrin degradation products

Diagram summarizing the known and possible functions of profilaggrin, filaggrin, and amino acids released by filaggrin proteolysis.



S. J. Brown, J Invest Dermatol. 2012



GRANULAR LAYER

Keratin aggregation → form macrofilaments

↓

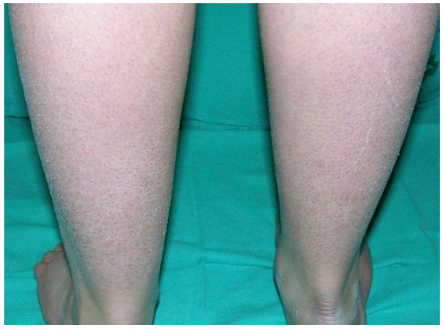
Filaggrin is degraded.

↓

Urocanic acid and pyrrolidone carboxylic acid

↓

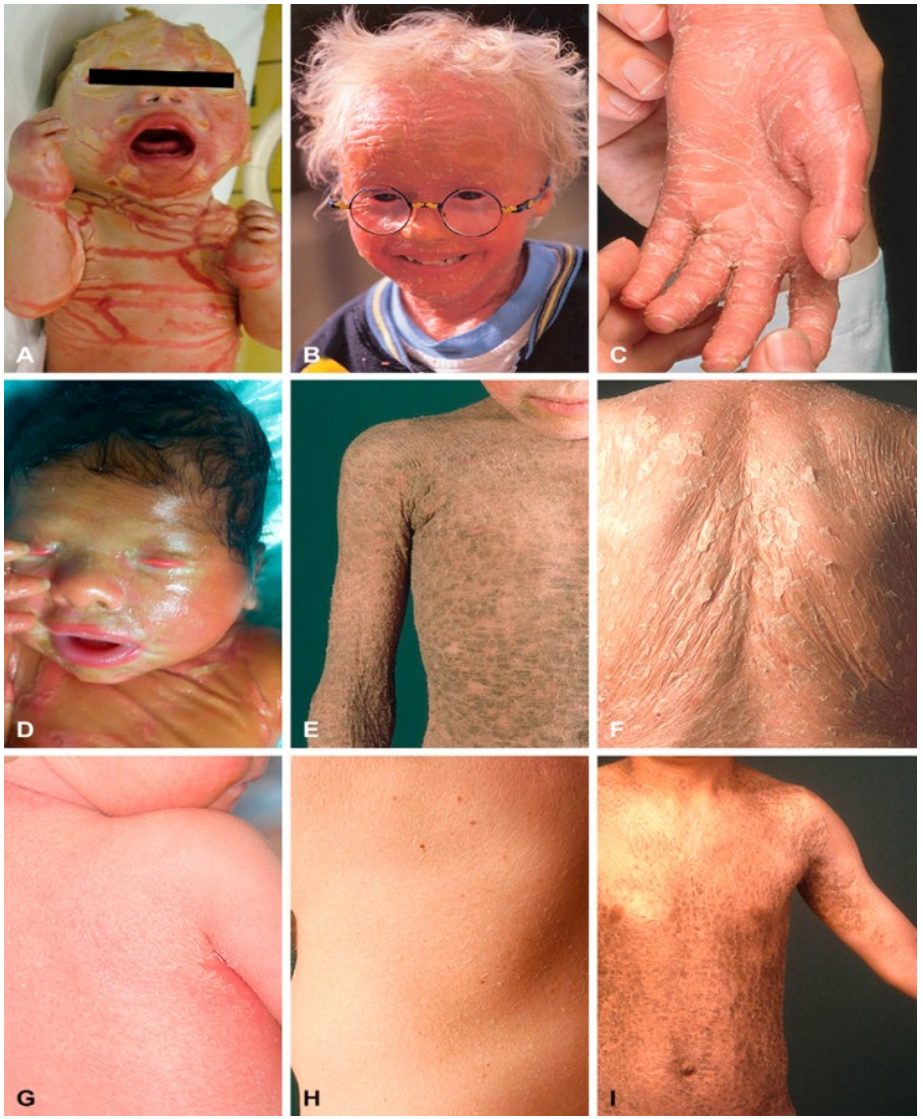
Contribute to hydration of the stratum corneum and help filter UV radiation



V. Oji, 2009

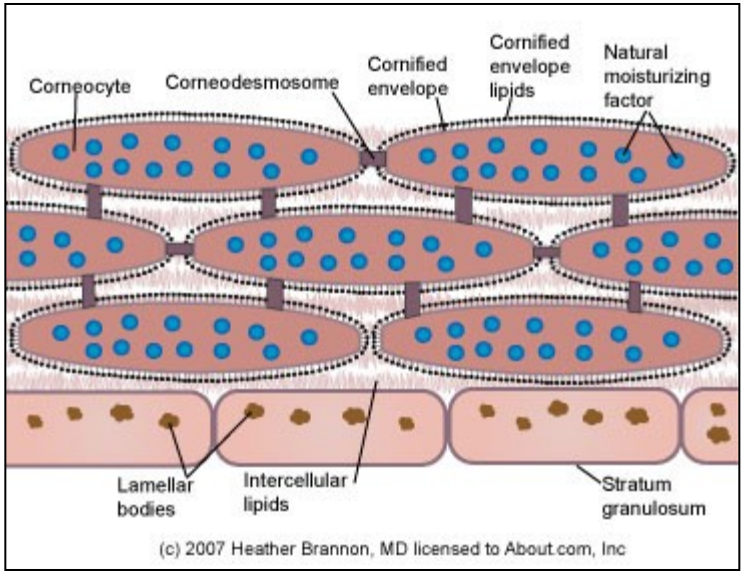
From 42 probands with ARCI:

- **14 (33.3%), *ALOX12B***
- **9 (21.4%), *ALOXE3***
- **7 (16.6%), *NIPAL4***
- **4 (9.5%), *CYP4F22***
- **3 (7.1%), *TGM1***
- **2 (4.8%), *ABCA12***
- 3 (7.1%) without causal mutations

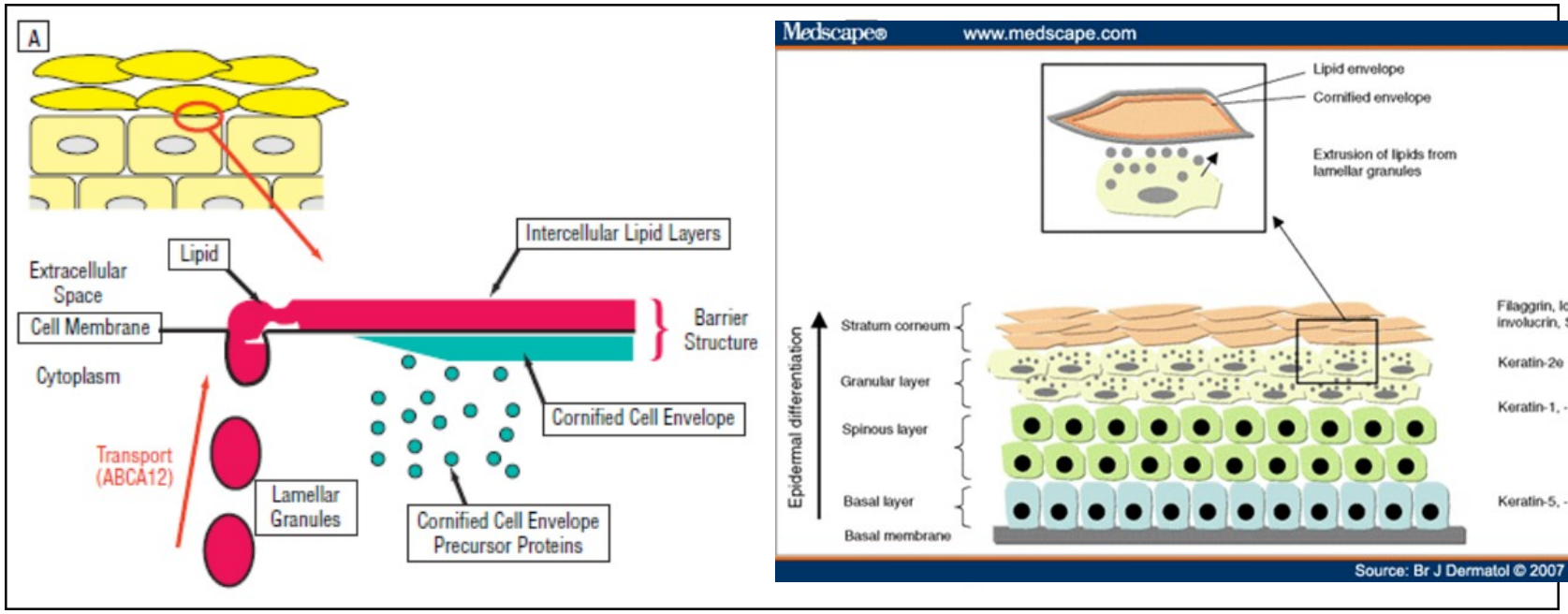


ARCI phenotypes: HI (harlequin ichthyosis), LI (lamellar ichthyosis), CIE (congenital ichthyosiform erythroderma)

- **Lipids** are packaged into **lamellar bodies** in stratum granulosum and then delivered by exocytosis to **intercellular space of stratum corneum**.
- After further conversion **lipids are organized into layers, constituting barrier** against water loss.

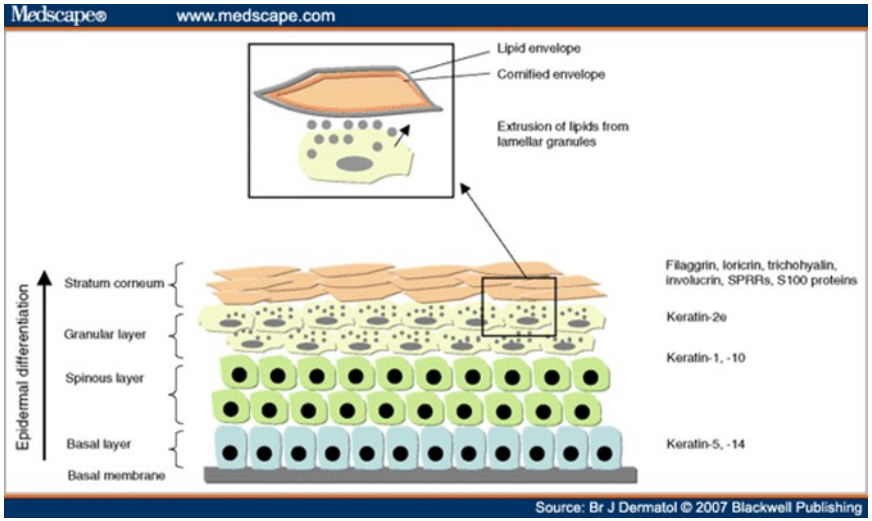


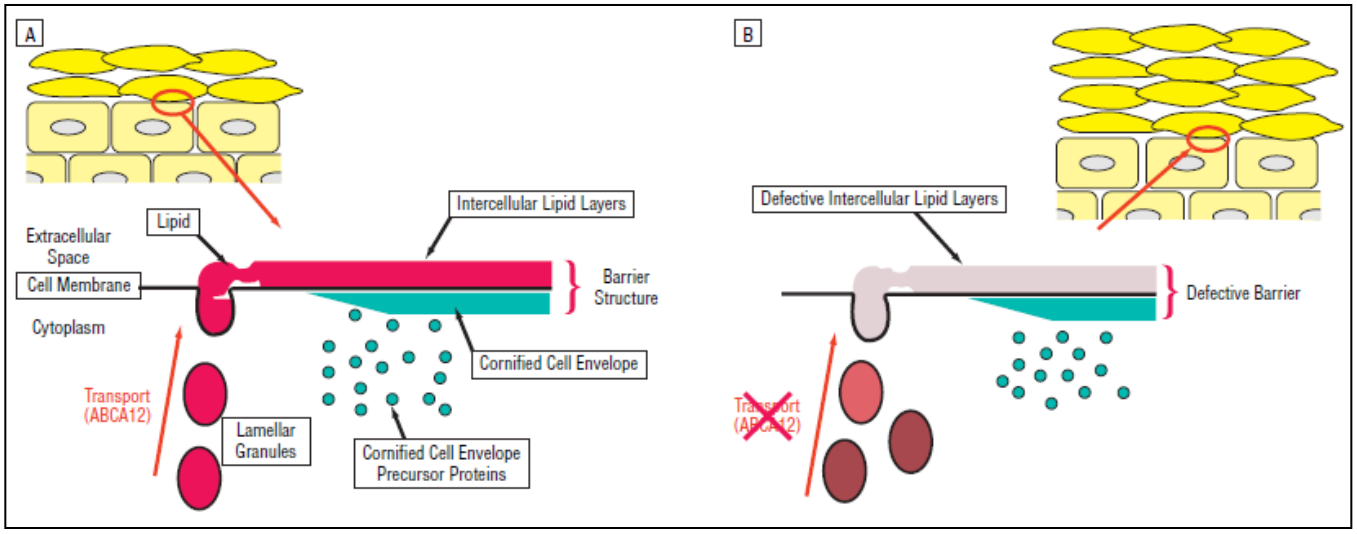
Akiyama M. 2006



Intercellular lipid layers in the stratum corneum, ABCA12

- **ABCA12 - keratinocyte transmembrane lipid transporter - transport lipids in lamellar granules**
- ABCA12 is a member of a large superfamily of ATP-binding cassette transporters, which bind and hydrolyze ATP to transport various molecules across a membrane.
- ***ABCA12* gene mutations underlie ARCI (HI, LI, CIE).**





Akiyama M. 2006

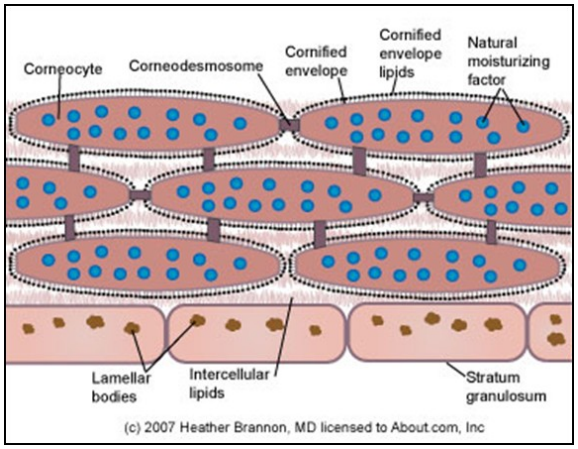
A, Model of formation of normal intercellular lipid layers and cornified cell envelope in stratum corneum.
B, **Loss-of-function mutations in ABCA12** lead to defective lipid transport via lamellar granules and **malformation of intercellular lipid layers**, resulting in loss of epidermal barrier function.

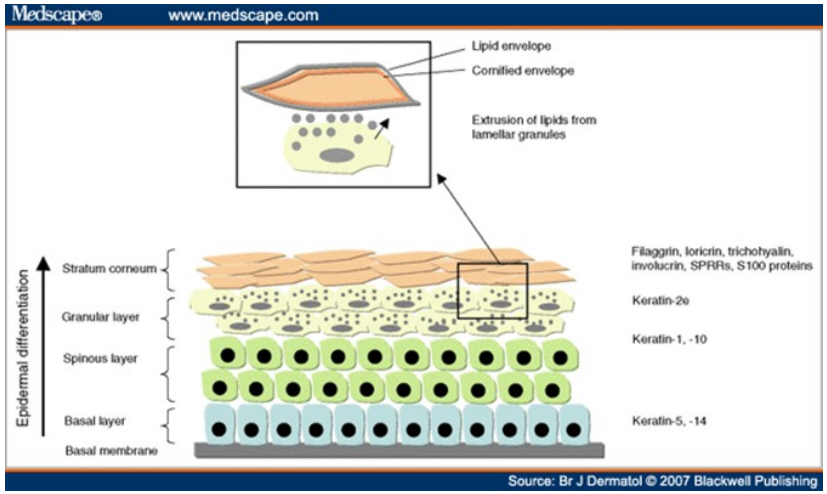
Intercellular lipid layers and cornified cell envelope formation:

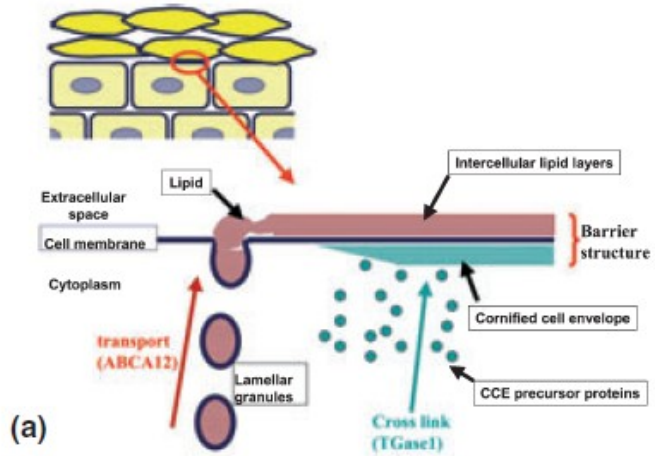
(a) Model of formation of the normal intercellular lipid layers and cornified cell envelope in the stratum corneum.

(b) ABCA12 dysfunction leads to defective intercellular lipid layers.

(c) Transglutaminase 1 (TGM1) deficiency results in a malformed cornified cell envelope and subsequent defective intercellular lipid layers.

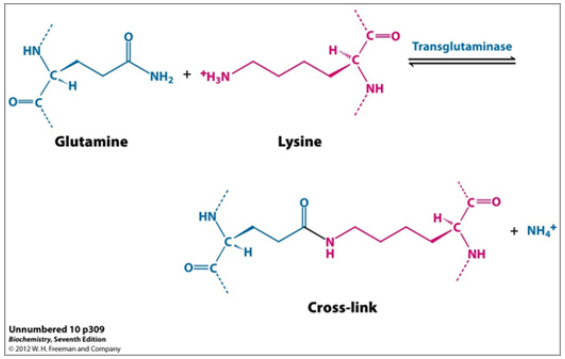


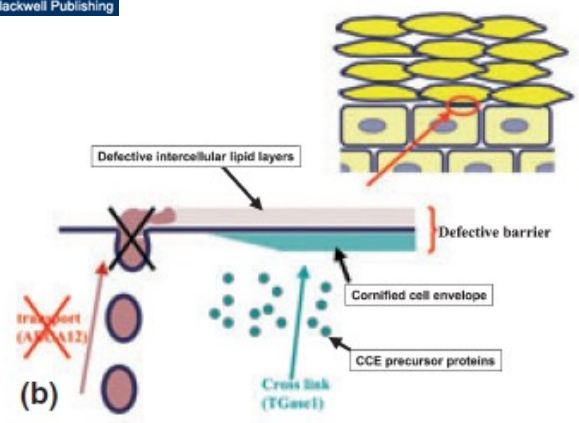


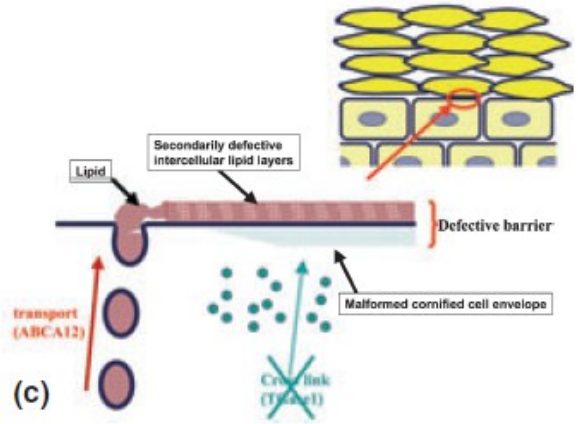


Akiyama M. 2006

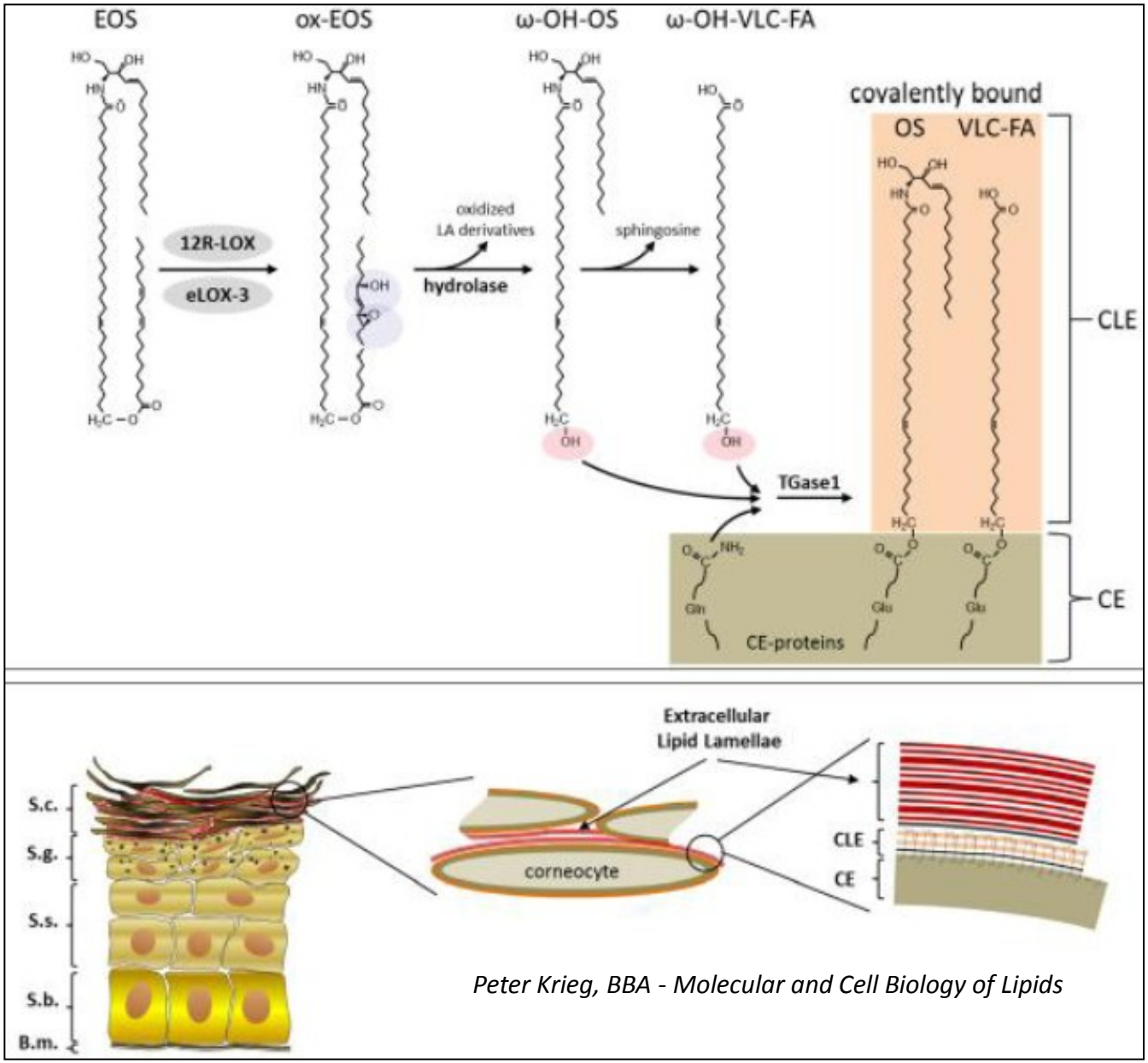
TGM1 function: cross-link of proteins







Lipoxygenase 12R (12R-LOX, *ALOX12B*), lipoxygenase-3 (eLOX-3, *ALOXE3*)



Peter Krieg, BBA - Molecular and Cell Biology of Lipids

- The oxygenation of **ceramide EOS → ω-hydroxyceramide (ω-OH-OS)**

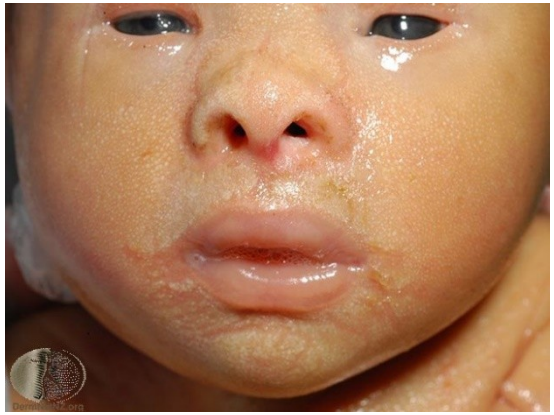
- ω-hydroxyceramide is further hydrolyzed to sphingosine and **ω-hydroxy-very long chain fatty acid (ω-OH-VLC-FA)**

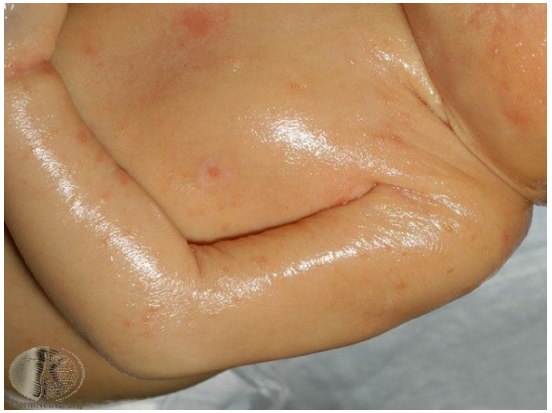
- **The ω-OH derivatives are crosslinked by TGM1 to glutamines of proteins of the cornified cell envelope (CE).**

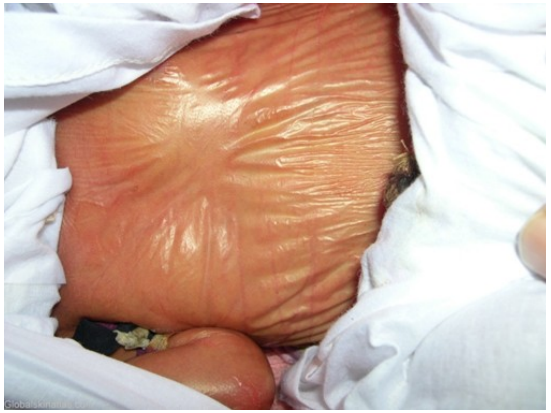
The corneocyte is surrounded by an inner protein (cornified cell envelope, CE) and an outer cornified lipid envelope (CLE), a lipid monolayer covalently bound to the CE.

EOS, O-linoleoyl-ω-hydroxyacyl-sphingosine; ox-EOS, 9R,10R-epoxy-11E-13R-hydroxylinoleoyl-ω-hydroxyacyl-sphingosine; ω-OH-OS, ω-hydroxyacyl-sphingosine (ω-hydroxyceramide); ω-OH-VLC-FA, ω-hydroxy-very long chain fatty acid; CE, cornified cell envelope; CLE, cornified lipid

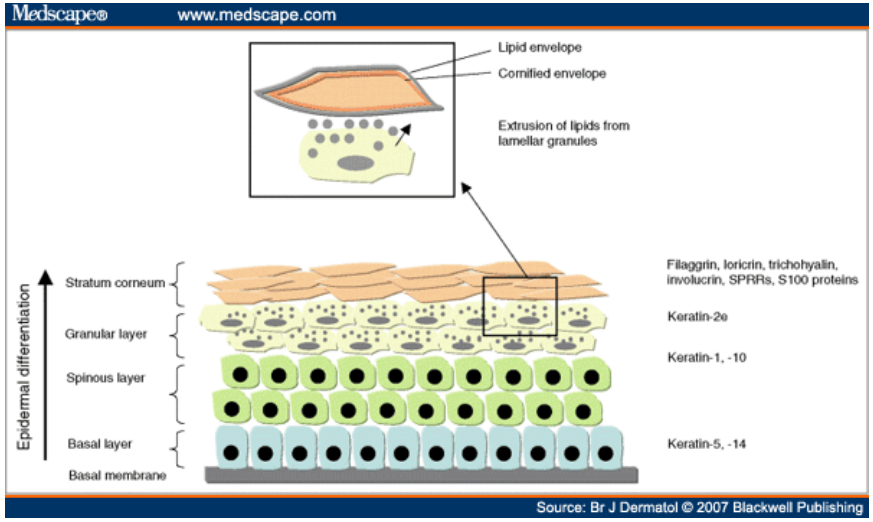
- ‘‘Self-healing collodion baby’’ or “self-improving collodion ichthyosis” – **the patients born as collodion babies upon shedding of the membrane acquire a nearly normal skin showing no or only mild signs of ichthyosis.**
- **The *TGM1* mutation p.Asp490Gly** – a chelation of water molecules locks the mutated enzyme in an inactive *trans* conformation under elevated hydrostatic pressure *in utero*; after birth, the water molecules are removed and the enzyme to isomerize back to an active *cis* form, explaining the dramatic improvement in the phenotype.
- **The *ALOX12B* mutation p.Tyr521Cys** – protein misfolding negatively affects the activity of mutated enzyme only under *in utero* conditions, thus explaining a dramatic improvement after birth.



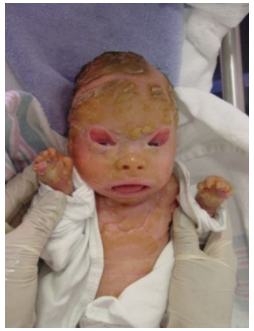


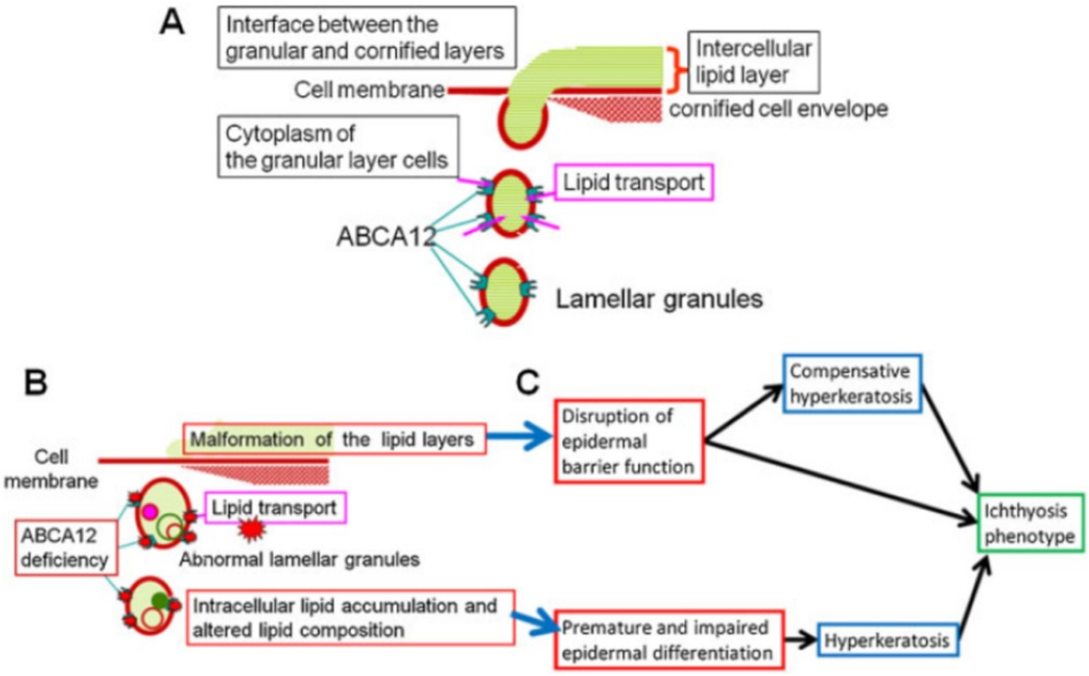


Intercellular lipid layers in the stratum corneum, ABCA12



Fetuses affected with HI start developing ichthyotic phenotype in amniotic fluid where stratum corneum barrier function is not required.

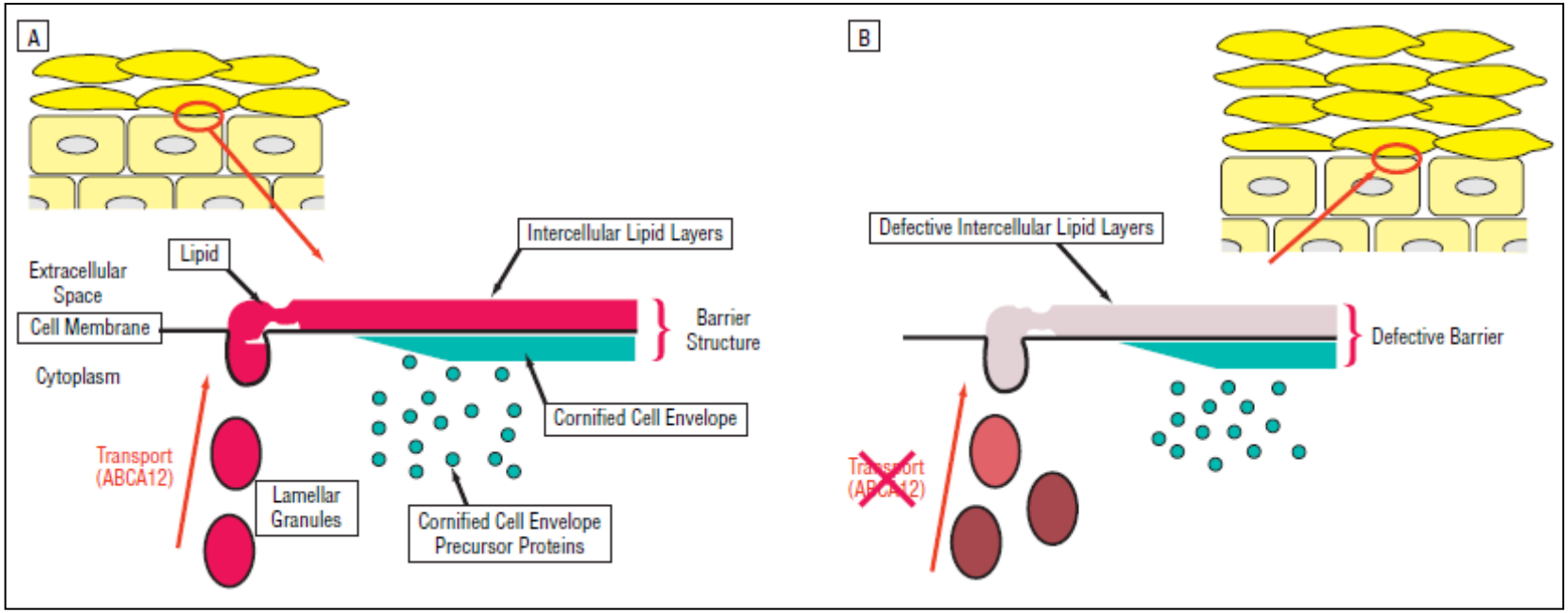




A: Lipid contents in LG are secreted to intercellular space forming intercellular lipid layers which are important for epidermal barrier function.
B: ABCA12 mutations disrupt lipid transport in LG and cause intracellular lipid accumulation.
C: **Disruption of epidermal barrier function and impaired epidermal differentiation** coordinately cause ichthyosis phenotype.

M. Akiyama, HUMAN MUTATION 2010

- **ABCA12 is a keratinocyte lipid transporter.**
- *ABCA12* mutations underlie 3 major clinical types - harlequin ichthyosis; congenital ichthyosiform erythroderma; lamellar ichthyosis (according to the type of mutations).



A, Model of formation of normal **intercellular lipid layers** and **cornified cell envelope** in the stratum corneum. B, **Loss-of-function mutations in ABCA12 lead to defective lipid transport via lamellar granules and malformation of intercellular lipid layers, resulting in loss of epidermal barrier function and abnormal hyperkeratosis.** *Akiyama M. 2006*

- **Collodion baby → congenital ichthyosiform erythroderma**

- **Sequence Capture and targeted resequencing**

- ***ABCA12*: c.5641C>T, p.(Arg1881*)**
- ***ABCA12*: c.69G>A, p.(Pro23Pro) ???**

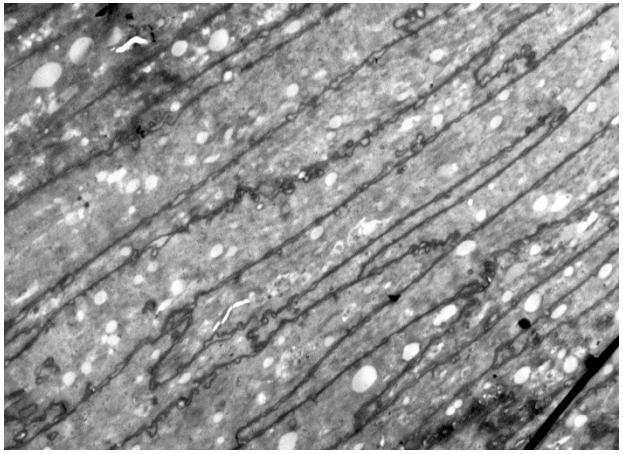
GAAGAGTTGATTGAGAAGTGCCTCTTGGTTAAGGATTAACCACA
GGGAAAAATCCAGCAGAAACAGAAGAACTGTGGGTTTCTTACCC
CAGCCCTCAAGGAAGCTATGCCGTGAAAGGGGTACTGATACACT
GACATACAGCAAGTTGGACGGGGCATCAGTTCTTCATTTGTGGA
GTGGAGAAAAGAAGAGGAAATCTCTCATTTGGGGCATTTGAAGG
ATGGCTTCCCTGTTTCATCAGCTTCAGATCCTGGTCTGGAAAAA
TTGGCTAGGTGTAAAAAGGCAGCC**G**gtgagttaaaaaaaagtg
tgggagtatgggtcatggggcaatacgccaggtaatcctaaaat
gtgatcttaatgagaagtgaaaacagaagagtttaaaggcattt
tccaaagcaggaagtaagatatttagaattcatccccctatctg
tcttatagctaatggagcgagtcttaacatgcccccaaaagcag
cctttt

Bioinformatic predictions of splice site scores in the mutation c.69G>A localised in the last nucleotide of *ABCA12* exon 1:

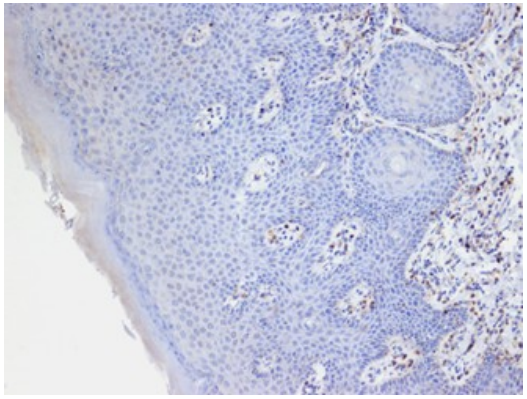
Nucleotide change	Sequence	MaxEnt Scan	NNSplice	NetGene2	HSF-MaxEnt	HSF-Matrices
WT	CCGgtgagt	11.64	0.99	0.67	10.9	94.09
c.69G>A	CCAgtgagt	8.33	0.58	0.58	8.28	83.51
% Change		**-28.56%**	**-41.41%**	**-13.43%**	**-24.04%**	**-11,24%**

performed by K. Veselý and M. Hermanová (FNuSA)

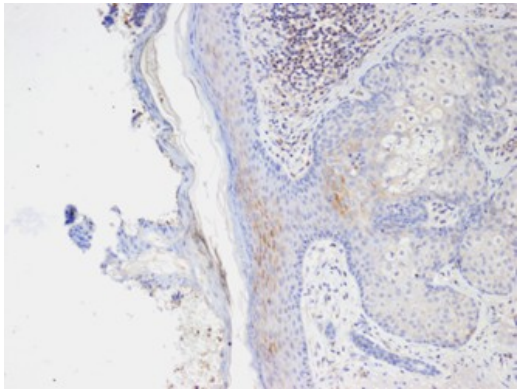
- **Ultrastructural analysis** (numerous droplets of neutral lipids in massively thickened stratum corneum)



- **Immunohistochemical analysis** of ABCA12 protein (**partial deficit of ABCA12)**



Patient skin tissue

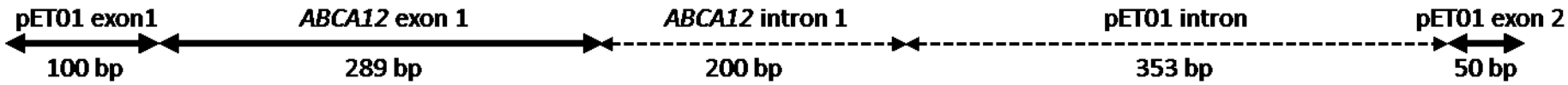


Control skin tissue

performed by K. Hrnčířová, P. Souček, T. Freiberger (CEITEC)

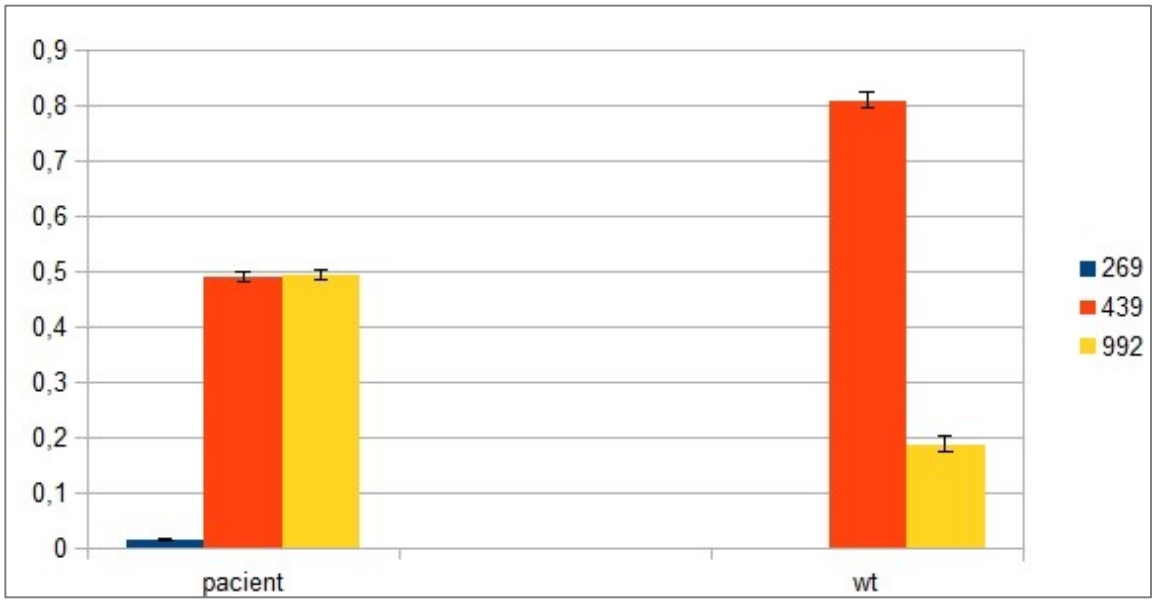
➢ Splicing mini gene assay

wt and mutant *ABCA12* exon 1 together with a part of intron 1 cloned into the pET01 vector



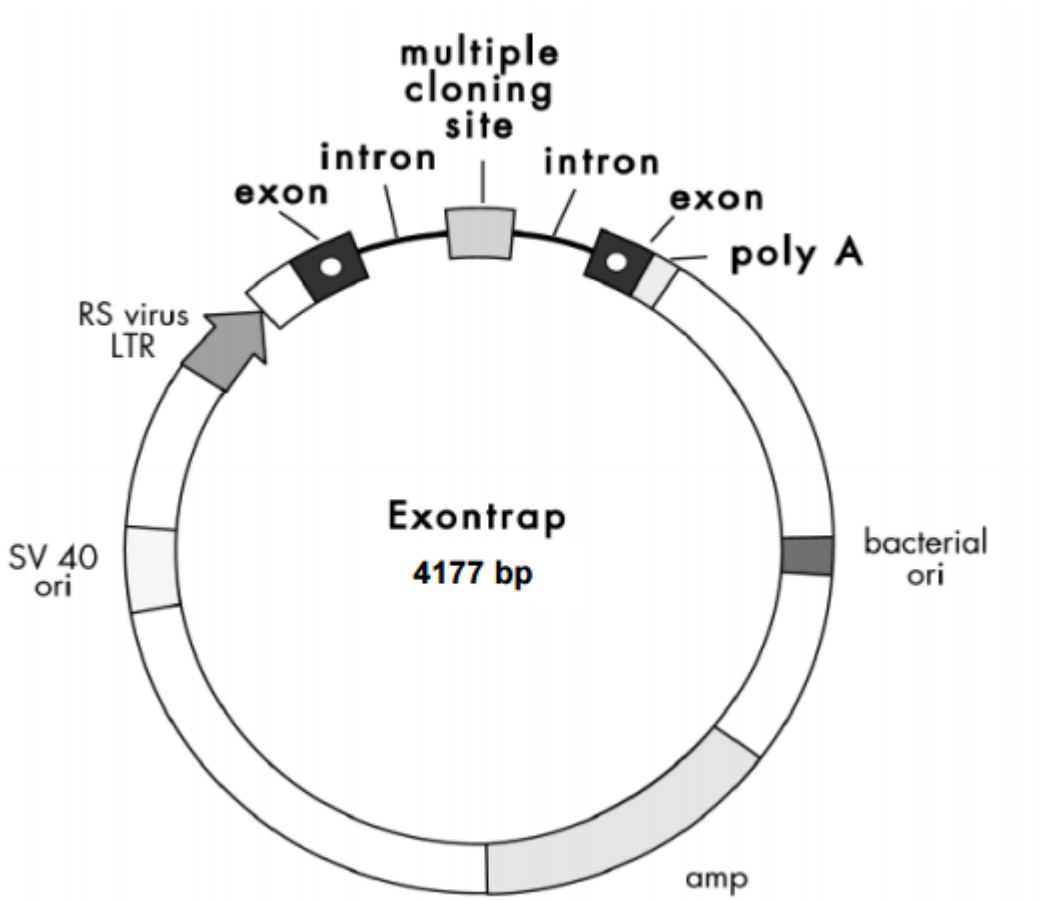
→ transfection of HeLa cells → analysis of splicing patern by qPCR

Results: **c.69G>A changes in a part of mRNA the splicing pattern – as a result of weakening of the 5´wt donor splice site**



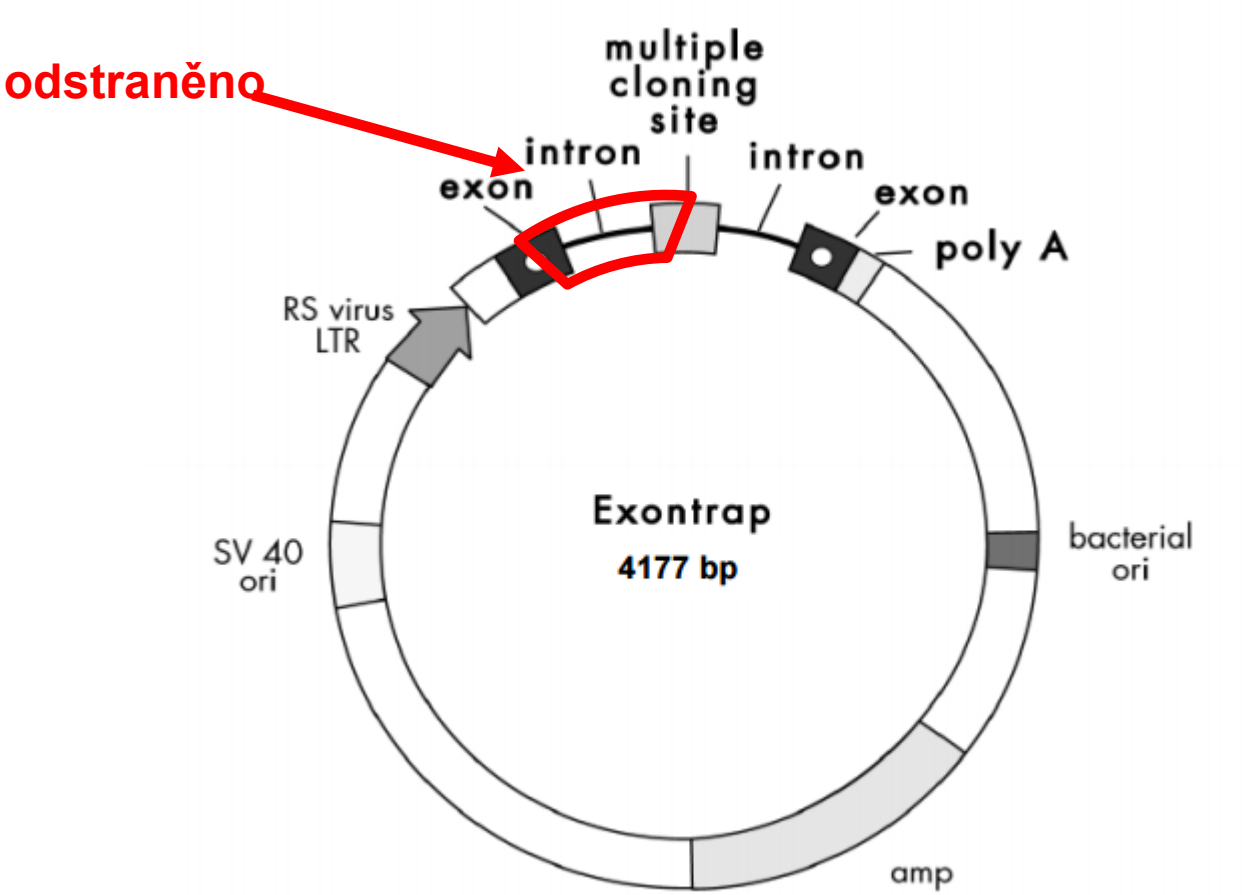
Úprava komerčního vektoru

- vektor pET01 Exontrap (MoBiTec)



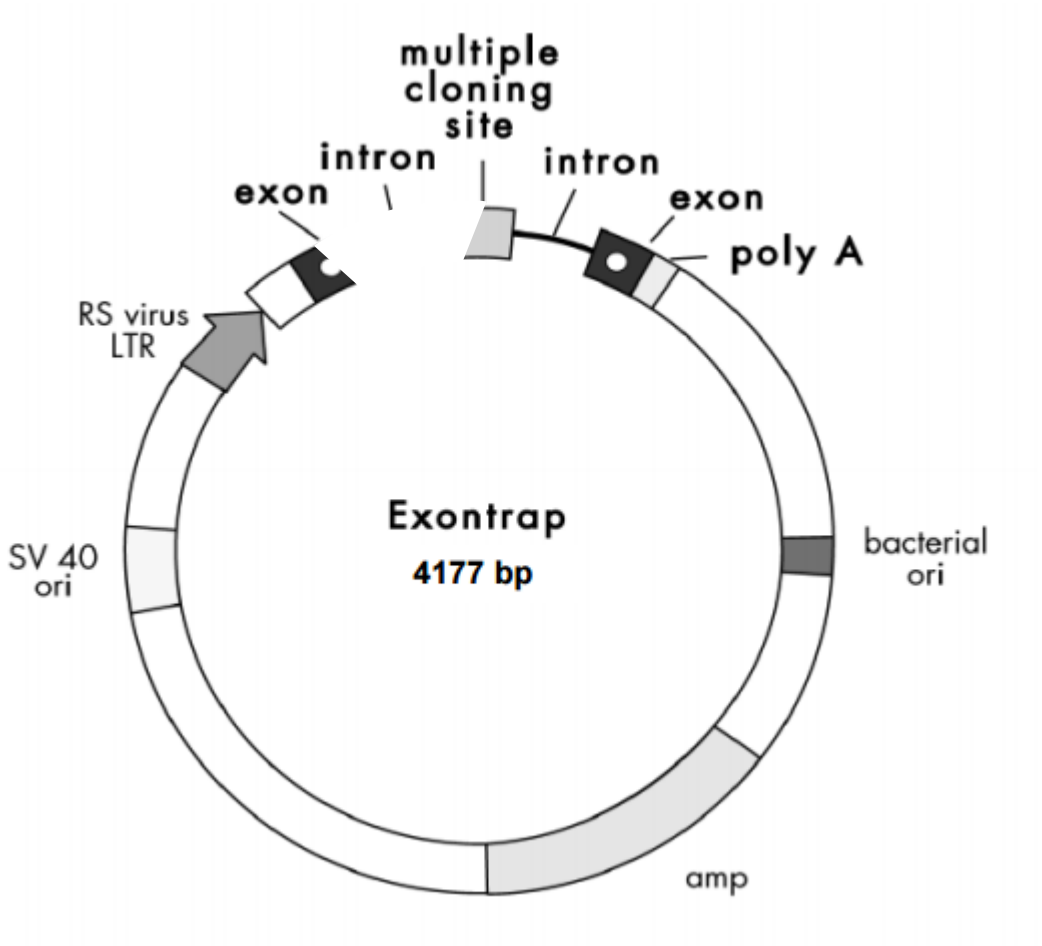
Úprava komerčního vektoru

- vektor pET01 Exontrap (MoBiTec)



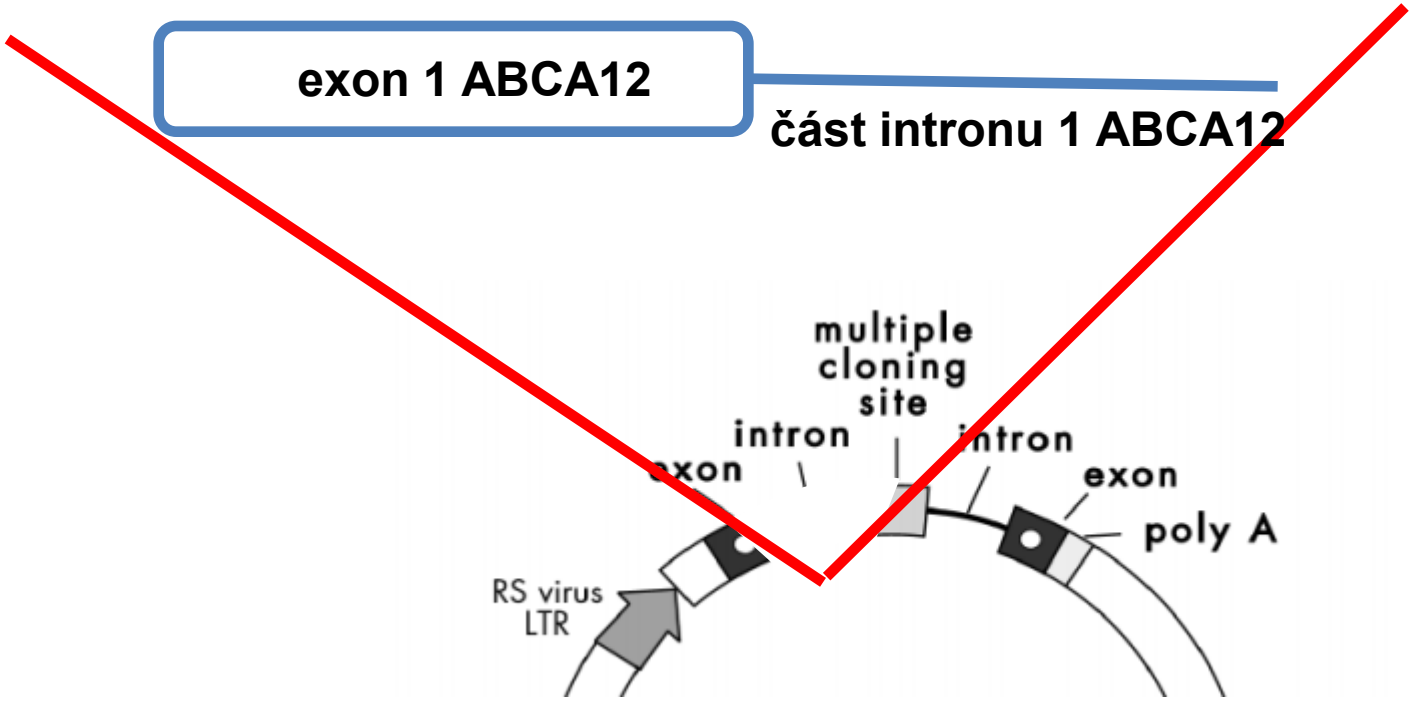
Úprava komerčního vektoru

- vektor pET01 Exontrap (MoBiTec)



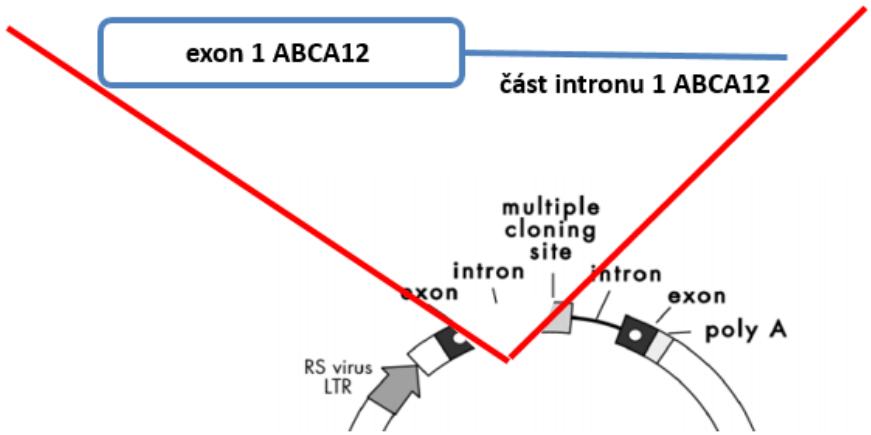
Úprava komerčního vektoru

- 1. exon genu *ABCA12* (napojen přímo na 1. exon vektoru pET) + část 1. intronu *ABCA12* (o délce 200bp)
- varianta pacient a wt



Úprava komerčního vektoru

GAAGAGTTGATTGAGAAGTGCCTCTTGGTTAAGGATTAACCACAGGGAAAAA
TCCAGCAGAAACAGAAGAACTGTGGGTTTCTTACCCCAGCCCTCAAGGAAGC
TATGCCGTGAAAGGGGTACTGATACACTGACATACAGCAAGTTGGACGGGGC **exon 1 ABCA12**
ATCAGTTCTTCATTTGTGGAGTGGAGAAAAGAAGAGGAAATCTCTCATTTGG
GGCATTTGAAGGATGGCTTCCCTGTTTCATCAGCTTCAGATCCTGGTCTGGA
AAAATTGGCTAGGTGTAAAAAGGCAGCC**G**gtgagttaaaaaaaagtgtggga **mutace G>A**
gtatgggtcatggggcaatacgccaggtaatcctaaaatgtgatcttaatga **intron 1 ABCA12**
gaagtgaaaacagaagagtttaaaggcattttccaaagcaggaagtaagata
tttagaattcatccccctatctgtcttatagctaatggagcgagtcttaaca
tgcccccaaaagcagcctttt

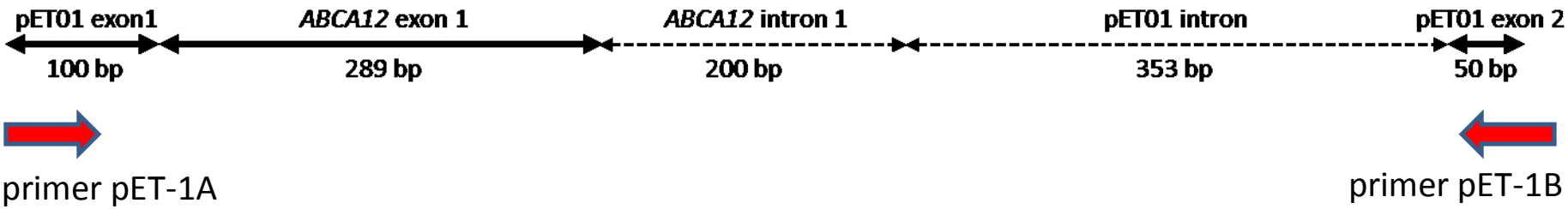


Aberantní sestřih mRNA

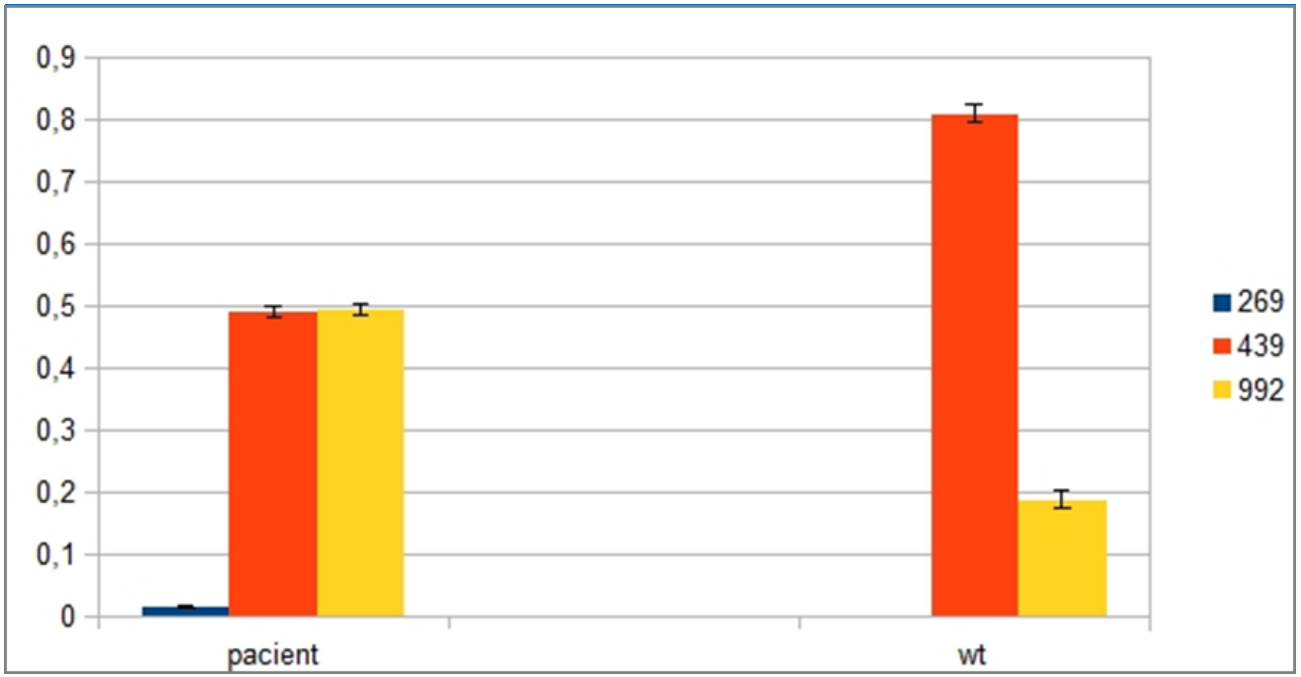
GAAGAGTTGATTGAGAAGTGCCTCTTGGTTAAGGATTAACCACAGGGAAAAATCCAGC
AGAAACAGAAGAACTGTGGGTTTCTTACCCCAGCCCTCAAGGAAGCTATGCCGTGAAA
GGG**G**TACTGATACACTGACATACAGCAAGTTGGACGGGGCATCAGTTCTTCATTTGTG
GAGTGGAGAAAAGAAGAGGAAATCTCTCATTTGGGGCATTTGAAGG**ATG**GCTTCCCTG
TTTCATCAGCTTCAGATCCTGGTCTGGAAAAATTGGCTAGGTGTAAAAAGGCAGCC**A**g
tgagttaaaaaaaagtgtgggagtatgggtcatggggcaatacgccaggtaatcctaa
aatgtgatcttaatgagaagtgaaaacagaagagtttaaaggcattttccaaagcagg
aagtaagatatttagaattcatccccctatctgtcttatagctaatggagcgagtctt
aacatgcccccaaaagcagcctttt

- červeně místo mutace – u pacienta **G na A** – poslední nukleotid 1. exonu ABCA12
- oranžově nově využité místo (po sestřihu zůstává z prvního exonu 119bp)
- modře – začátek translace proteinu ABCA12
- podtržené – vystřižená oblast exonu 1 při využití kryptického místa (nejkratší PCR produkt o velikosti 269bp)

Výsledný konstrukt:



- Transfekce HeLa buněk, kultivace
- Izolace RNA (24 hodin po transfekci)
- Reverzní transkripce, semikvantitativní PCR, vyhodnocení



Nesetřižená mRNA

Správně setřižená mRNA

Aberantně setřižená mRNA